UNIVERSITY of TASMANIA

DETERMINING THE EFFECTS OF NUTRIENT ENRICHMENT ON MACROALGAE-DOMINATED REEFS (OBSERVATIONAL, EXPERIMENTAL AND PREDICTIVE CAPABILITIES)

by

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Declaration of originality

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Abstract

The impacts caused by chronic nutrient enrichment on coastal habitats usually comprise a slow stepwise progression of chemical and biological changes which can be highly influenced by the physical environment. As a consequence, the process of change is dependent upon the unique environmental circumstances in each system and detection of impacts, particularly in the early stages, can be difficult. In addition, the influence of multiple anthropogenic nutrient sources interacting at a community or species level further complicates impact assessments. However, understanding the mechanism by which increasing changes in nutrient availability might affect the complex nature of the coastal reef ecology is essential for early detection, subsequent prevention and control of impacts.

The capacity to predict how natural variability interacts with anthropogenic stressors is a challenge for both marine scientists and environmental managers. Natural selective forces in costal habitats (e.g., light levels, wave exposure, salinity, temperature and species interactions) will influence the degree to which reef systems respond to sources of nutrients. However, these forces can also promote the system's ability to cope with impacts (resistance) or to recover from a given disturbance (resilience), and will vary spatially and temporally throughout geographical gradients within the same system. Understanding the system characteristics and key biological responses may help to determine where potential impacts may or may not take place.

This study outlines a field experiment, which measurably increased the nutrient availability in three reef systems to determine changes in macroalgal community composition, successional stages and classical indicators (i.e., fast-growing opportunistic species). In addition, this study examined how alternative indicators, including underlying indicators of impact such as physiological sensitivity of key macroalgal species), can provide better indication of the status of impact/ stress. Furthermore, location-specific variations of abiotic factors were monitored to test if physical drivers, the structuring forces of gradients in community structure, may improve our understanding of eutrophication.

The results indicate that there was no evidence of major effects of nutrient enrichment on the overall community structure and that the observed responses of opportunistic species were not consistent. The abundance of opportunistic species differed between locations and showed significant effects of small-scale variability within each community. Physical drivers (variations in wave exposure and light, as well as differences in salinity and temperature) were correlated with fundamental differences in the established and the successional community structure throughout the study region. This suggests that fundamental and prevailing components of the community (e.g., Caulerpales/Fucales and/or Encrusting algae/Fucales-dominated reefs) and abiotic fluctuations may underpin opportunistic algae abundances and define the responses to enhancement of nutrients. This has important implications for our previous understanding of nutrient enrichment indicators and how this relationship might be applied to management (i.e., where the presence of fast-growing opportunistic species in abundance is indicative of an adverse environmental

impact) - as in many cases this interaction may simply represent a difference resulting from prevailing environmental conditions.

The spatial effects of diverse sources of nutrient (i.e. sewage treatment plants, marine farming, catchment and river inputs) could be differentiated by analysing the nutrient tissue composition (C, N, P) in key algal species, and was to a lesser extent evident in their photosynthetic performance, which may require further research given the limitation that the assessment of Chlorophyll-a fluorescence may have in macroalgae from nutrient limiting waters. These findings suggest that the physiological responses of key algal species may constitute a more reliable indicator of system status. Since some species are likely to reach their nutrient requirements at low/moderate nutrient concentrations (e.g., fucoids) they may be good at long-term storage of limiting nutrients that may otherwise mask species responses to nutrient variation and/ or addition (Pedersen 1995). For this reason it is also suggested that macroalgae on particular reefs may be pre-adapted to different nutrient inputs depending on the prevailing nutrient regime.

In conclusion, these results highlight that in a real life scenario the usefulness of classical indicators of eutrophication may be constrained by location-specific gradients and small-scale spatial variability. Spatial patterns in the biophysical environment (e.g., wave exposure, light environment) indicated gradients in community structure and habitat attributes that in turn would appear to restrict eutrophication responses in macroalgal reefs. Inclusion of tissue nutrient levels and photosynthetic performance of key species in assessments of

eutrophication appeared to better define the spatial role of physical drivers (like wave exposure and light), and sources of nutrient availability. Incorporating this information into ecosystem models may improve the analytical power.

This study provides important data to improve spatial management in systems with macroalgal reefs and suggests that characterisation of the physical environment is essential to contextualise the system response and that evaluation of the sensitivity of key species at a physiological level may provide a more accurate and effective indication of the impact due to nutrient enrichment in coastal habitats than the determination of localised increases in opportunistic species.

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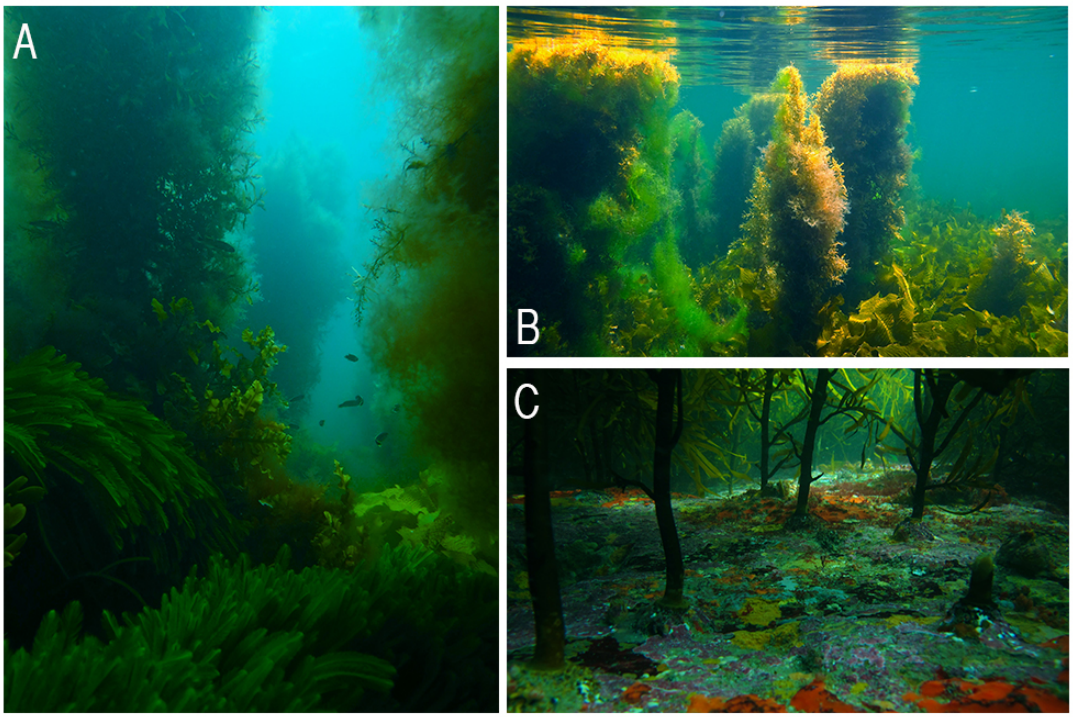

Rocky reefs habitats dominated by macrophytes around Tasmania probably embody one of the most outstanding landscapes of the temperate coastal zones of the world. With an extraordinary marine diversity this environments exhibit great number of endemic species of plants and animals. Unfortunately, human stressors such as global warming, CO_2 intensification, sea-level rise, pollution and in particular, land and sea based nutrients may alter the natural equilibrium that maintains these ecosystems. In the photo, rocky reefs habitats from The D'Entrecasteaux Channel SE Tasmania. (A) Tinderbox Marine Reserve, (B) Green Island and (C) Ninepin Point Marine Reserve. Photos A and C by Luis Henríquez and B by Lucy Quayle.

1. Chapter 1. Rocky reefs under stress of nutrient enrichment

1.1. Introduction

The marine plant communities that surround continental and insular coastlines extend in a narrow band from approximately 2 to 40 m depth, covering an area of between 2,000,000 – 6,800,000 km^2 (Kennedy, 2010) and represent a essential biotope in our oceans. Macroalgal dominated reefs provide the structural foundation for a diverse range of marine communities; they constitute breeding areas and food source and refuge for invertebrates, fish and marine mammals, and as such underpin the multiple functions of coastal ecosystems. In addition, macroalgal communities can play an important role in reducing sediment accretion and coastline erosion providing a coastal resilience mechanism to the negative effects of sea-level rise, as a result of wave action (Duarte et al. 2013b). These habitats also play a critical role in regulating climate change - they are important sinks for carbon, able to sequester and store three times the levels of Carbon that can be incorporated by equivalent areas in terrestrial ecosystems (McLeod et al. 2011). However, being in the transitional area between land and sea these habitats are also extremely vulnerable to human activities and are arguably amongst the most impacted marine ecosystems worldwide (Scheffer et al. 2001, Halpern et al. 2008). Significant loss of macroalgal habitat as a result of human induced pressures has already been widely reported (Krause-Jensen et al. 2008, Thornber et al. 2008, Gorman et al. 2009). There are a great many ways in which these coastal reef systems can be adversely impacted; activities on land associated with industrialisation and urbanisation can affect environmental flows to the coastal region and introduce a range of

pollutants, agricultural and aquaculture development can add nutrients whilst water based activities such as fishing can result in pressures on biodiversity and affect species interactions and ecosystem stability (Worm et al. 2006b, Johnson et al. 2011). Understanding the potential impacts of such pollutants on reef ecosystems is key to developing risk appropriate management and monitoring strategies.

Nutrient enrichment in coastal waters is one of the most significant threats to coastal reef ecosystems (Russell et al. 2005, Krause-Jensen et al. 2008, Teichberg et al. 2010). Nutrients enhancement can arise through leaching of land-based fertilizers either directly or via manure for agriculture and animal husbandry. Fertiliser usage has increased markedly from 32 to 88 million tonnes per year from the early 70s to 2005 (Smil, 2002; Duarte 2013). Marine aquaculture production is perhaps a more direct source of nutrient enrichment into coastal areas and has also increased markedly over the last ten years, from 2.2 to 33 million tonnes per year (FAO, 2013). These anthropogenic nutrient inputs can change the natural balance and availability of essential nutrients (i.e. Nitrogen and Phosphorus) for primary producers, which can lead to significant changes in the primary productivity in coastal seas (Dixon et al. 2014, Paerl et al. 2014). This in turn can manifest in increases in the abundance of “nuisance” macroalgal blooms (Duarte et al. 2013a), toxic phytoplankton (Hallegraeff 2010) and the frequency of bottom oxygen depletion (Nixon et al. 2001, Painting et al. 2007). Catastrophic changes in canopy-forming macroalgae have been reported from highly populated areas (Gorgula & Connell 2004, Worm & Lotze 2006a, Halpern et al. 2008). Recovery strategies have been implemented in a number of cases (Painting et al. 2007) but clearly it would be preferable to have strategies in place to try that would enable management actions to be implemented before the situation deteriorated to such a level. There is a clear need for

reliable monitoring approaches that could give an early warning of the potential for ecosystem changes.

1.2. South Australian reef threatened by nutrient anthropogenic enrichment

The accelerated development of agriculture and aquaculture with their incidental nutrient inflows to marine environments has been particularly accelerated at temperate latitudes (Nixon 1995). One of the major concerns is that marine ecosystems from temperate waters present high abundance of endemic species (Edgar 2008). For instance, temperate rocky reefs of southern Australia are a major reservoir for biodiversity, equivalent to that found in tropical reefs and with higher endemism than their tropical counterpart (Kerswell 2006, Edgar 2008). This level of endemism makes these reefs highly sensitive to impact and therefore ensuring that management controls are appropriate is critical (Bennett et al. 2016). The kelp *Ecklonia radiata* (Laminariales, Lessoniaceae) is dominant in these environments, with kelp habitat covering approximately 71,000 km^2 along Southern Australia coasts (Bennett et al. 2016). This habitat provides a range of valuable ecosystems services such as tourism, fisheries and food, and supports global climate change protection and biodiversity (Duarte et al. 2013b, Bennett et al. 2016). Overall, 71% of Australia's population live in temperate coastal areas, and the associated anthropogenic pressures will inevitably present risks for these marine ecosystems.

In the last century the estuarine coastal habitats of New South Wales (e.g., Sydney Harbour), Victoria (e.g., Port Phillips Bay), South Australia and The Derwent estuary in Tasmania have all been shown to have clear symptoms of coastal degradation due to historical pressure of urbanisation, industry

development, agriculture and land use (Birch 2000, Johnston & Keough 2002, Townsend & Seen 2012, Stuart-Smith et al. 2015). Studies to specifically investigate the effects of nutrient enrichment in southern Australia have indicated that nutrient-driven degradation has promoted shifts from kelp forests to turf-algae-dominated landscapes in a number of areas (Kennelly 1987, Gorgula & Connell 2004, Connell et al. 2008, Connell et al. 2014). These impacts, seems to have more severe consequences in oligotrophic waters (Russell et al. 2005) where mechanisms to regulate turf-algae proliferation (e.g., grazing control) seem to be controlled by the combined effect of ambient nutrient availability (i.e., oligotrophic vs. eutrophic) and the nature of the nutrient load (i.e., short-term vs. long-term inputs) (Russell & Connell 2005, 2007). The occurrence of underwater deforestation (i.e., turf forming algae dominated habitats) seems to be more frequent where the difference between the anthropogenic nutrient input and the ambient nutrient concentration is greatest (Gorman et al. 2009). This suggests that the inherent characteristics of the particular system are critical in determining the scale of any impact. This also implies, that some ecosystems/ habitats may have a potential for resilience and/or resistance to anthropogenic impacts and that this potential may be related to their inherent biodiversity or functional redundancy (Wernberg 2010 , Downing et al. 2012). Consequently, location-specific responses to nutrient enrichment may be a way to define the intrinsic susceptibility of that environment to anthropogenic nutrient additions. If this is the case then preventive strategies to changes of diversity may be worth considering in coastal management and environmental policies.

1.3. Tasmanian rocky reefs

Coastal reefs extend along a major part of the 2,833 km of the Tasmanian coastline (Geoscience Australia 2016). They underpin important fisheries resources such as the southern rock lobster (Jasus edwardsii) and the green and black-lip abalone (*Haliotis rubra* and *Haliotis laevigata*, respectively) (Johnson et al. 2011, Hinojosa et al. 2014). Coastal reefs in Tasmania are exposed to a variety of anthropogenic stressors. These include urban pollution, a range of nutrient inputs derived from agriculture, forestry, animal husbandry and salmon farms, as well as, oceanic uptake of atmospheric carbon dioxide (Connell et al. 2013) and ocean warming (Hobday & Pecl 2013). In recent decades frequent southward incursions of warm-poor water of the Eastern Australian Current have increased transport of the sea urchin *Centrostephanus rodgersii* larvae, extending its range of distribution. These urchins have had a devastating effect on the coastal ecology of the regions in which they occur, turning productive reef habitat and populations of the giant-kelp *Macrocystis pyrifera* into "urchin barrens" – bare rock area with little or no ecological value (Ling 2008, Ling & Johnson 2009). This has resulted in "cascade-effects" - altering the distribution and ranges of fish and invertebrates and compromising the overall integrity of shallow macroalgal communities in eastern Tasmania (Johnson et al. 2011). In such a changing environment, the effect of these human-caused stressors combined with the increasing action of anthropogenic nutrient may have complex effects on key macroalgal species threatening the entire algal community structure within reef habitats.

Eutrophication is defined as "the increase in the rate of supply of organic matter to an ecosystem" (Nixon 1995) and as such can have a major impact on the function and structure of reef ecosystems. This in turn may have

negative economic consequences for users such as, fisheries, tourism and aquaculture (Smith et al. 1999, Worm et al. 2006b). In recent years Tasmania has become a worldwide important producer of Atlantic salmon (Buschmann & Muñoz, 2016 *in press*). The intensive culture system where tens of thousands of fish are cultured in floating cages has raised environmental concern because its intrinsic capacity of altering the nutrient availability of surrounding waters and impact benthic biodiversity (Crawford et al. 2003, Macleod et al. 2004, Edgar et al. 2005, Macleod et al. 2008). The intensification of dissolved inorganic nutrients released by salmonids aquaculture can increase the occurrence of macroalgal blooms (Buschmann et al. 2006, Buschmann et al. 2009), with negative effects on the community structure of coastal reefs (Oh et al. 2015). Consequently, adaptive ecologically based management needs to minimise potential risks associated with coastal development and aquaculture. Monitoring that is sensitive enough to provide an early warning, in a timeframe where management actions can be instigated, is an important prerequisite in this management process.

1.4. Evaluation and Monitoring

In recent decades the development of effective tools for monitoring eutrophication has been the focus of both local and international governmental organizations, particularly in regions that have already shown chronic symptoms of eutrophication such as Europe, United States and China (Painting et al. 2007, Bricker et al. 2008). Current thinking in terms of environmental monitoring runs along three lines, which can be used in combination or independently. These include establishing: 1) Threshold values for rapid evaluation of environmental deterioration - such as trophic

status of aquatic systems (i.e. oligotrophic, mesotrophic, eutrophic and hypertrophic) and water quality (turbidity). 2) Empirical relationships to define how changes in reef communities from eutrophied waters and systems with low nutrient concentrations relate to other environmental parameters such as chlorophyll, light attenuation (Krause-Jensen et al. 2008) and size of the nutrient source (Gorman et al. 2009). 3) Predictive numerical models – these can be simple tools relating nutrient concentration and increases in chlorophyll or more complex models that incorporate physico-chemical and biological variables and effects (Gowen et al. 1992, Nixon et al. 1996, Baretta-Bekker et al. 1997, Painting et al. 2007).

Threshold values can provide extremely useful limits for management and some of the more common thresholds applied for eutrophication in coastal systems relate to chlorophyll levels, phytoplankton biomass, and light attenuation (Krause-Jensen et al. 2007a). However, these are all generally related with water quality and do not provide a specific indication of reef health; there are no specific threshold values that define macroalgal condition. Defining empirical relationships between environmental condition and reef health would clearly provide a useful tool for monitoring and management. However, those relationships that have been described tend to differ spatially, i.e. within regions and between ecosystems (estuaries, embayments and coastal lagoons) (Gowen et al. 1992, Krause-Jensen et al. 2008). This suggests that relationships tend to be location specific. This will also affect how we use predictive modelling, as any model would have to be able to incorporate this spatial specificity. Models would need to be developed for each individual system, and verified in the field to account for any unique sources of spatial variability (Nixon et al. 1996, Baretta-Bekker et al. 1997, Painting et al. 2007). At the moment although there are some models capable of predicting changes in water column nutrients, we do not

yet clearly understand the relationships between these environmental conditions (nutrient inputs) and reefs communities well enough to build models that could reliably predict changes in reef conditions. Studies that relate environmental conditions to reef performance are needed to underpin the development of predictive models.

Threshold values often relate to measurable changes in an environmental parameter along a gradient e.g. measuring factors like oxygen level, temperature or a particular nutrient, with specific values indicating a step change in condition. Species changes within a system may also be considered indicators of a step change in condition. As mentioned earlier, a common symptom of eutrophication is the proliferation of fast-growing algae (Valiela et al. 1997b, Gorgula & Connell 2004, Russell et al. 2005), these species can be indicative of the initial stages of ecosystem deterioration that may lead to subsequent replacement of complex climax communities and as such could be considered an ecological threshold. However, it is difficult to predict the point of occurrence (timing) and scale of such environmental changes. Numerous field and laboratory observations have shown that community changes caused by nutrification and opportunistic macroalgae may not respond to gradual increases in nutrients, but to spatial-specific environmental differences in stepwise abrupt community shifts (Valiela et al. 1997a, Schramm 1999, Cloern 2001, Kraufvelin et al. 2006, Krause-Jensen et al. 2008, Gorman et al. 2009). In addition, synergistic relationships with other stressors can create non-linear responses making it difficult to establish causal relationships (Cloern 2001). Once again, this suggests that empirical studies relating macroalgal response to differences in environmental condition and nutrient load are needed if we hope to identify meaningful indicators of eutrophication.

Indicators of nutrient enrichment need to be able to distinguish both acute, high impact episodic events but also the chronic long/ medium-term cumulative effects that may determine “likelihood” of impact. One example is the use of manipulative/ comparative nutrient-dose response experiments, which are able to evaluate spatial differences in ambient nutrient limitation (Teichberg et al. 2008, Teichberg et al. 2010). Similarly, the determination of the nutrient status comparing inherent differences between anthropogenic sources and ambient nutrient availability has constituted a useful management tool providing a spatial context of habitat sensitivity (Gorman & Connell 2009, Gorman et al. 2009). Indicators able to distinguish spatial differences in high, moderate and low level nutrient enrichment can be key to establishing associated risks for coastal habitats. Moreover, they will provide a clear understanding of the system status and those elements, which are protective of the carrying capacity at a level that is relevant for identification of system degradation in realistic timeframes for management. It may not be possible to address all of these factors with a single indicator, and it may be that a suite of complementary measures is necessary. In addition, indicators that are able to identify different levels of sensitivity (i.e. different thresholds) or differences in nutrient availability would greatly improve our ability to evaluate and manage ecosystem changes.

The coastal ecosystem in southeast Tasmania provides an excellent environment to test these issues (Figure 1.1). Salmon aquaculture in Tasmania has grown steadily from 15,208 tonnes in 2003 to 42,707 tonnes in 2013 (FAO, 2012). Approximately 50% of this production takes place in a semi-sheltered estuarine system in southeast Tasmania, The D’Entrecasteaux Channel and the Huon estuary (pers. comm. TSGA 2015).

The D’Entrecasteaux Channel and the Huon estuary is a microtidal salt-wedge estuary system, which has strong similarities with estuaries elsewhere in Australia and temperate zones. The system is well mixed and includes

several sources of anthropogenic nutrients; such as nine wastewater treatment plants, catchment inputs from agriculture, forestry, livestock and fish farms (Ross & Macleod 2013). The hydrodynamics of the system suggest a predominantly northward circulation (Figure 1.1), which has been shown to influence the dispersion and the residence time of nutrients throughout the system (Butler 2006, Herzfeld et al. 2008). In addition, there is an environmental gradient both in terms of anthropogenic inputs and natural influences that allows testing of the relevance of site specificity (spatial scales) on any empirical relationships and threshold values.

It is important when establishing the relationships between changes in environmental conditions (nutrient impacts) and changes in macroalgal communities that any assessments are undertaken at a scale that is consistent with impacts in the real world. So far, there are few studies which have considered realistic nutrient scales or responses at multiple sites, and this limits the extent to which the findings can be used to infer real life scenarios of change (Cloern 2001). Furthermore, modelling approaches based on small-scale experimentation may be difficult to scale to broad-scale responses and field scenarios (Thrush et al. 1997, Thrush et al. 2000, Wernberg et al. 2012).

The objective of this study was to test spatial differences in the response to nutrient loads in macroalgal community structure and key macroalgal species by manipulating the nutrient regime at three naturally varying field locations. The results will be used to better inform management and monitoring of nutrient inputs and impacts on the temperate rocky reef habitats of southeast Tasmania.

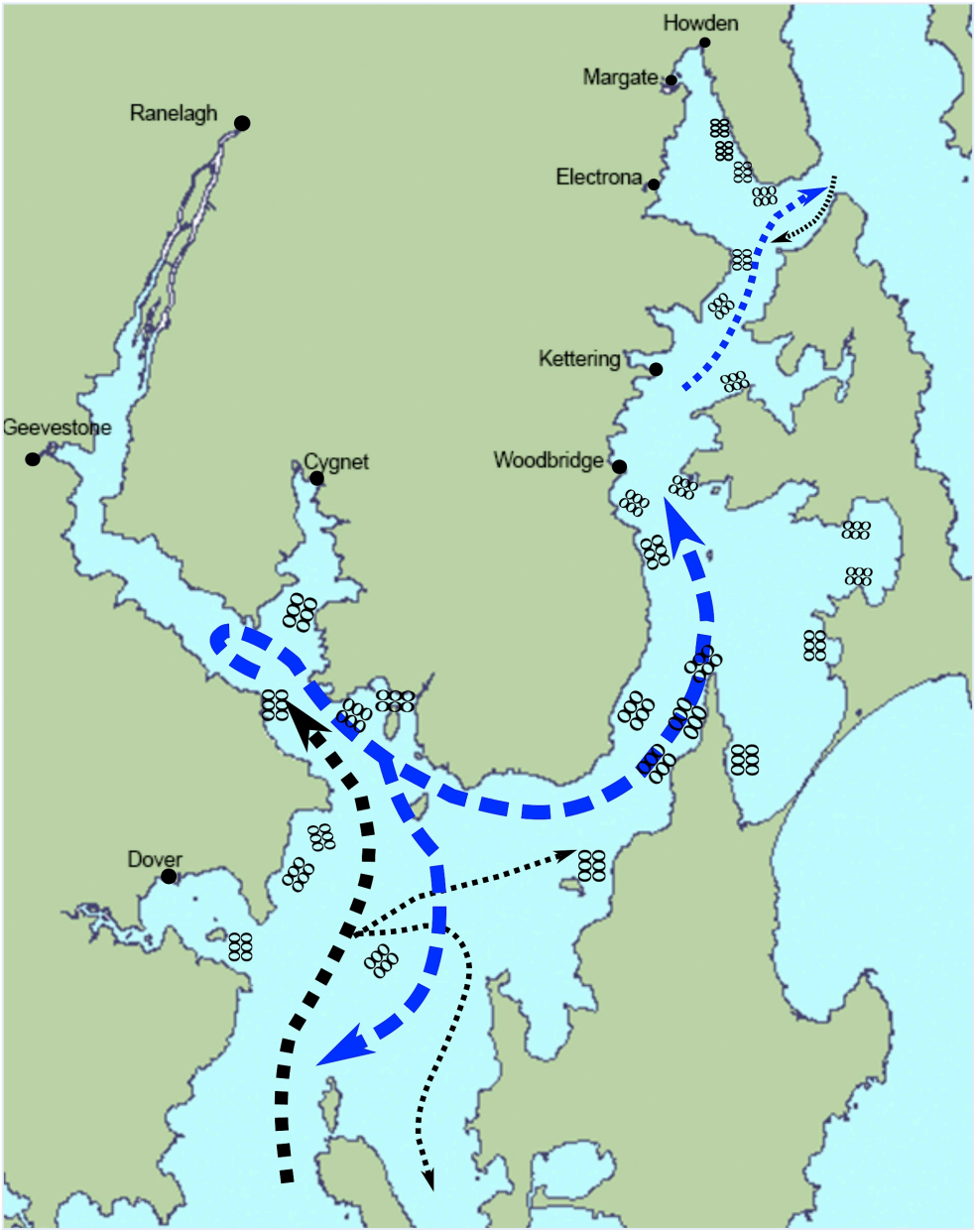

Figure 1.1. Map of the D'Entrecasteaux Channel and Huon region showing main patterns of surface circulation (blue arrows), bottom currents (black arrows), and the location of key nutrient inputs - Waste Water Treatment plants (●) are indicated by the site name listed and salmon farms are shown as clustered circles. (Source: Ross & Macleod 2010; DPIPWE, 2015)

2. Chapter 2. Nutrient-dose response of opportunistic species of macroalgae as indicator of the eutrophication process on rocky reef communities

2.1. Introduction

Potential catastrophic changes in coastal habitats as a result of marine eutrophication are a big concern in temperate coastal areas (Kinney & Roman 1998, McGlathery 2001, Korpinen et al. 2007a, Connell et al. 2008, Duarte et al. 2009). Coastal reefs represent a critical habitat for the reproduction and recruitment of many commercially important species, and provide a refuge and food source for mammals, fish and invertebrates (Worm et al. 2000, Worm & Lotze 2006a, Barrett et al. 2009, Johnson et al. 2011). In addition, vegetated coastal habitats have been shown to play an important role in mitigating the adverse effects of sea-level rise by providing a buffer zone which decreases coastal erosion and sediment accretion (Duarte et al. 2013b). Also, these habitats provide a major Carbon sink because algal canopies and seaweed beds can capture sediments rich in C transported from riverine and oceanic sources (McLeod et al. 2011). Macroalgal reefs play a critical role in maintaining ecosystem health. Eutrophication represents a major threat to these communities. Consequently, it is important to understand what indicators we could use to manage that threat.

Unfortunately, we still don't understand how different reef systems respond to nutrient enrichment effects, or how the intrinsic biophysical variation might influence individual reef systems (Russell & Connell 2007, Krause-Jensen et al. 2008, Duarte et al. 2009). In addition the combined effects of multiple environmental stressors may increase the negative effects (Wernberg et al.

2012). The early effects of eutrophication on reef condition, before any effects on carrying capacity or clear shifts in coastal communities are evident, can be subtle and hard to identify. Understanding the early indicators of nutrient impacts and how these might drive biological change is a challenge for marine scientists and coastal managers (Cloern 2001, Krause-Jensen et al. 2008, Connell 2008), but one that needs to be resolved.

One well established effect of eutrophication is the proliferation of bloom-forming macrophytes (Thornber et al. 2008, Lyons et al. 2014); in extreme cases these algae may cover hundreds of kilometres (e.g., *Enteromorpha prolifera* green-tide; Liu et al. 2009) with serious ecological effects in marine communities. Experimental evidence has shown that community changes caused by nutrient enrichment tend to manifest as a stepwise process of sudden shifts, rather than a gradual modification of community structure as nutrient concentrations increase (Schramm 1999, Bokn et al. 2003, Kraufvelin et al. 2006). Where these opportunistic species occur in abundance they can compromise the viability of the habitat-forming macro-algae (Sand-Jensen & Borum 1991, Duarte 1995, Pedersen 1995, Valiela et al. 1997b, Cloern 2001, Kraufvelin et al. 2002, Russell et al. 2005). Under extreme conditions this can lead to loss of habitat and coastal ecosystem services, and may even compromise the overall resilience of the ecosystem (Valiela et al. 1997b, Kinney & Roman 1998, Scheffer et al. 2001). In SE Tasmania the D'Entrecasteaux Channel supports approximately 50% of the Australian salmon farming industry and as such inputs a significant nutrient load into this system (pers. comm. TSGA 2015). To date there does not appear to have been any major structural change in rocky reef communities in this region but there have been subtler signs of nutrient enrichment near fish farms, specifically evidence of increased abundances of opportunistic macrophytes (Oh et al. 2015), which might suggest that the system is under

stress. This highlights that it is important to identify not only the nutrient load that would drive primary community change but also the levels at which these lesser effects are initiated, as these may be the indicators that would allow for management action before irreversible damage occurs.

Several studies have conducted small-scale nutrient-dose experiments to try to establish the biological response of reef systems to eutrophication. These studies have shown that variability in physical factors such as water motion and wave action, salinity gradients and light attenuation (Bokn et al. 2002, Kraufvelin et al. 2002, Bokn et al. 2003, Eriksson & Bergstrom 2005, Kraufvelin et al. 2006, Kraufvelin 2007, Wernberg & Connell 2008) can play a large part in determining the sensitivity of the system to eutrophication and to the proliferation of bloom-forming algae. Whilst, these experiments have provided valuable insights into the underlying mechanisms driving macroalgal community responses they still cannot fully explain the variability in the system (Krause-Jensen et al. 2008).

Field experiments have shown that habitat-forming species, such as Laminariales and Fucales (Eriksson et al. 2006a, b, Worm & Lotze 2006a, Eriksson et al. 2007, Wikstrom & Kautsky 2007), and consumer pressure (Eriksson et al. 2006a, b, 2007, Russell & Connell 2007, Connell et al. 2011) play a key role in influencing a particular community's response to nutrient enrichment. In addition, experimental evidence have shown that greater disparity in resource availability between the total human nutrient inputs and the ambient nutrient levels will have more critical ecological effects on macroalgal reefs (Gorman et al. 2009). However, a meta-analysis of the overall effect of macroalgal blooms on ecosystems showed that the effects may be highly variable (Lyons et al. 2014), and in some circumstances may even increase biodiversity (Thomsen & Wernberg 2015). These results would

tend to suggest that each system is unique, and as such overall patterns in cause-effect relationships might be difficult to establish. Thus, differences at regional scales may bring insights about particular system capabilities to respond to nutrient inputs. However, this leads to the question – could there might be groups of reef or conditions that could respond in similar ways to eutrophication?

Coastal reef ecosystems comprise a wide variety of environmental gradients. Geographical features that create dissimilar patterns of circulation may in turn result in dissimilar levels of nutrient retention and assimilation in the associated primary producers (Martins et al. 2001, Nixon et al. 2001, Herzfeld et al. 2008). Thus, the identification of natural physical gradients may provide a proxy reflecting fundamental differences in reef ecosystems and their ability to cope with anthropogenic nutrient loads (Schramm 1999, Cloern 2001, Krause-Jensen et al. 2008). In this study we will examine how the random variability in physical conditions might influence opportunistic algae proliferation associated with nutrient enrichment.

Spatial variation in physical factors such as ambient nutrient levels, wave action, light, salinity and temperature may underpin the resilience of particular regions. Therefore, it is important to understand and describe both the physical and biological characteristics as these may represent a proxy for sensitivity/ resilience and would therefore be a useful factor for consideration in coastal management decisions.

Field experiments and broad-scale observations will provide a better understanding of the system's ability, and in particular the macroalgal community's ability, to cope with increases in nutrient availability under real world conditions. In this study, we undertook a realistic field based nutrient

load experiment in which we increased loads by at least two orders of magnitude above those reported for previous marine fertilization studies on shallow rocky reefs. The early response of classical indicators (i.e., opportunistic macrophytes and macroalgal community structure) and potential differences in prevailing physical factors were tested on three reefs communities to identify the potential effects of a moderate impact scenario.

We tested the hypothesis that the response of community structure and opportunistic algae to changes in nutrient availability is directly related to the location of the reef system and the prevailing biophysical conditions. So, we determined to what extent spatial variations of prevailing environmental factors (light, wave exposure salinity, temperature and nutrient regimes) could explain any observed response. We also tested the reliability of opportunistic algae as direct indicators of nutrient inputs and community change. Ultimately, this will provide insights into how geographical differences in the physical environment may constrain the response of macroalgal communities to increased nutrient loading, as well as, into understanding the causes of current patterns in algal assemblages in southeast Tasmania.

2.2 Methods

2.2.1 Area of study

The D'Entrecasteaux Channel and Huon Estuary are situated on the western side of Bruny Island and southeast Tasmania and form an expansive and interconnected estuarine system (Figure 1 A). The dominant surface flow originates near the southern limit of the D'Entrecasteaux Channel where the Huon River joins waters coming in from the Southern Ocean, creating a general flow to the north throughout the Channel (Herzfeld et al. 2008). The system has an average flushing rate of 20 days (Butler 2006). However, patterns of water transport associated with seasonal changes of the microtidal regime, influence the residence time ranging from 3-5 days in the lower Huon estuary and North West Bay, to 13 days in Isthmus and Great Bay at the mid Channel area (Herzfeld et al. 2008). High nutrient loadings entering the southern end of this system during winter are generally associated with the convergence of nutrient-rich sub-Antarctic waters coupled with freshwater inflows from the Huon River. In addition, occasional incursions of the Zeehan current can bring down warmer, nutrient-poor waters from the Indian Ocean. During spring/summer the system tends to be dominated by nutrient-poor subtropical waters from the Pacific Ocean transported by the Eastern Australian Current (Cresswell 2000, Ross & Macleod 2013). In this sheltered system support the production of 27,000 tonnes per year of Atlantic salmon (ABARES 2013), which constitutes approximately the 50% of the Australian salmonids production. Other significant anthropogenic sources of nutrients include local wastewater treatment plants, carrying residential and industrial effluents. Along with this

catchment inputs and agricultural runoff across the entire Channel constitute the major sources of nutrients inputs in this region (Ross & Macleod 2013).

2.2.2. Water column enrichment

Ambient nutrient conditions were manipulated in three shallow rocky reefs by adding 12 mesh bags (1mm mesh size) containing approximately 17 kg of coated fertilizer Osmocote-Plus® (19% of Nitrogen, 6% Phosphate, 9% soluble Potash, 7% of Sulfur and 1% of Iron, plus trace compounds) starting in autumn (April). Each mesh bag was attached to a concrete mooring. These nutrient supply units (henceforth supply units) were deployed haphazardly within a 100m^2 plot, with the plot itself indicated by ropes attached to the bottom (Figure 1 B, C and D). This experiment provided 80 kg of nitrogen per treatment plot, which based on an industry standard Food Conversion Rate of 1.4 (Mente et al. 2006) is comparable to the amount of nitrogen contained in feed for the production ca. 900 kg of Atlantic salmon. This amount of fertilizer provides 64 kg of combined phosphates, soluble Potash and sulphur and 8kg of Iron (see product chemical composition table, *www.everris.us.com*).

A release rate model was used to estimate the longevity of the resin-coated pellets under field conditions (Samuel Stacey – Technical Manager Everris Ltd., April 2013). As nutrient release is dependent on temperature, coated pellet dissolution (and hence nutrient release) was estimated based on the maximum and minimum surface water temperature of the D'Entrecasteaux Channel for 2011. This model indicates that theoretically between 22-24 weeks approximately 60% of the nutrient supply would be released into the surrounding waters (Figure 2). However, release rates are expected to be fast in seawater and local scale fluctuations in water motion/ wave exposure

and temperature may also influence the release performance (McGlathery 1995, Worm et al. 2000). Previous marine fertilization experiments using this method have suggested that release rates in seawater declined after 6-8 weeks, with most of the pellets having been dissolved by that time (Lotze et al. 2000, Worm et al. 2000, Worm et al. 2001). However, these previous experiments used much smaller volumes of fertilizer, in the current study the amount of fertilizer used was more than two orders of magnitude greater which would greatly increase the dissolution time. In addition the supply units were regularly checked and fertilizer replaced whenever they appeared to be less than half full. This resulted in supply units being deployed in autumn (April) and/or replaced after 20-24 weeks (between five to six months) in spring (October). According to this the plots should be enriched for a period > to 10 months ensuring a sampling campaign during winter, spring and summer of around nine months.

Nutrient concentrations were measured in water samples taken at 10-15 cm from supply units, following prior experimental procedures using coated-fertilizer and agar solutions (McGlathery 1995, Lotze et al. 2000, Worm et al. 2000, Teichberg et al. 2008) and additionally with samples taken at 1 m from the supply units.

Three control plots with similar topography and depth were delimited at each location (Figure 1 B, C, D). Multiple controls were included in the experimental design specifically to distinguish any confounding effects of natural variability from the effects of nutrient additions on natural assemblages (Hurlbert 1984, Underwood 1991, Chapman et al. 1995). Because of limited available reef area at similar depth, not all sites were of equal size; one control area was delimited as a 100m^2 plot and the two remaining as 60m^2 plots. The study locations ranged between 1.5 to 2.5 m depths (from low tide mark), and covered a lineal distance of approximately

200 m along the coastline. At each location the treatment plots were separated ca. 50 m away from control plots, which in turn, were spaced ca. 30 m. In this parcels, 15 permanent quadrats of 30 × 30 cm were set up within each of the 100 m^2 plots, and 9 quadrats were set up within each of the 60 m^2 control plots. Each quadrat was randomly displayed within a radius of 1 m from the supply units.

2.2.3. Monitoring of macroalgal communities

Macroalgal percentage of cover was estimated using a 30 × 30 cm point-contact quadrant with 25 regularly distributed contact points approximately every three months since autumn to summer. After the cover of canopy-forming algae was assessed these were moved aside to enable quantitative determination of the underlying understory species. At each sampling time five independent quadrats in the 100 m^2 plots were assessed, three quadrats in the 60 m^{-2} plots were assessed and all species were removed for species identification.

Macro-algae were identified to the highest practicable taxonomic level. Abundance estimates were tabulated by species and additionally classified into functional groups. Species were separated into easy-to-identify groups based on a visual determination of the prevailing structural characteristic they serve within their community. The intention was to assess a quantitative response of a morphologically diverse group of functionally similar species (e.g. opportunistic-fast growing macroalgae) but also species that may present very similar morphologies (e.g. Caulerpa species). This classification also considered the works of Littler & Littler (1980) and Steneck & Diether (1994), which provide a clear definition of different groups based on algal morphology, environmental productivity and stress, and survival strategy (Littler & Littler 1980, Steneck & Dethier 1994). However, some modification of these functional separations was applied in order to obtain a broad

generalization of the potential effect of nutrient additions on the highly diverse algal assemblage of SE Tasmanian (Table 2.2).

Uniseriate filamentous (e.g *Cladophora*), single layered green foliose algae (e.g. *Ulva*), corticated filamentous algae (e.g, *Polysiphonia*) were grouped together as nutrient enrichment indicators. Densely packed small thick and thin filamentous algae (< 2 cm tall), commonly referred to as turf-forming species (Connell et al. 2014) were also included in this group. Multilayered, corticated foliose algae (e.g., Zonaria) and corticated macrophytes (e.g. Plocamium), usually growing under canopy-forming species were classified as understorey species. Leathery large brown algae with erect thallus were separated as Fucales (e.g., *Phyllosphora comosa*, *Sargassum* spp.) and Laminariales (e.g., *Macrocystis pyrifera, Ecklonia radiata*). Erect articulated calcareous and encrusting forms that cover the substrate with a thin film, commonly known as Lithothamnion species (Steneck & Dethier 1994, Edgar 2008) were separated as Encrusting/Coralline algae. All mat-forming species of Caulerpa due to its unusual branches linked to horizontal stolons, which rapidly occupy substrate, were grouped in a single functional group (Table 2). An initial estimation of the abundance for each group was carried out in autumn 2012, prior to Osmocote addition; this provided the base-line macrophyte community composition for each location.

2.2.4. Monitoring of environmental variables

2.2.4.1. Wave exposure

Wave exposure levels were determined from each plot. “Openness” indices were generated using the Generic Relative Wave Exposure Model (GREMO) implemented within the ArcMap™ program in ArcGIS™ 9.3 (ESRI product)

according to the methods referenced in Hill et al. (2010). This program uses the geographical location to calculate effective length for wind action and distance to any mass of land blocking access to the open ocean (GEODATA, Coast 100K 2004, Geoscience Australia). The index was calculated as the sum of the length of the 48-fetch lines constructed from a point at the center of each plot from every location. The data was subsequently normalised by dividing by the maximum possible sum of fetch lengths. Additionally, 'Bathymetry-altered fetch' was generated to account for the attenuation of wave energy (wave friction with seafloor) with depth. The main assumption of this model is that a wave has the same probability to approach to a point from any direction. Although this index is not a direct assessment of the mechanical effect of the wave action it provides accurate information about the probable state of the exposure to wave according geographical and physical features (e.g., wind).

2.2.4.2. Nutrients

Dissolved inorganic nutrient concentrations were monitored every three months at all locations. Water samples (n = 3) were collected using 60 ml syringes at 10 cm and 1m from the supply units in treatment plots, and randomly across control plots. The samples were filtered on site using GF/ F 0.45-µmol glass fibres to reduce contamination through ammonia excretion from zooplankton, and then placed in a chilled container until they could be frozen. Samples were sent to the Water Studies Centre at Monash University for chemical analysis of ammonia (NH_3), nitrate + nitrite (NOx) and phosphate (PO_4). Nitrogen compounds were determined using flow analysis injection with filterable-reactive method (FIA) with an injection analyzer Quick-Chem 8500 (Lachat Instruments, Colorado, U.S.A).

2.2.4.3. Salinity and Temperature

Variations in salinity (PSU) and temperature (C°) were measured using conductivity loggers (HOBO® U24) deployed at each site, installed in the centre of each treatment plot. Similarly, the light levels were monitored through HOBO loggers, which were cleaned of fouling using an automatic brush (sweep of light sensor every 20 minutes). All the devices were deployed at ~1m from the bottom attached to moorings from late winter to summer within the treatment plots at each location.

2.2.5. Statistical analysis

2.2.5.1. Multivariate

The analyses of macroalgal community structure and the associated abiotic factors were undertaken using PRIMER 6.0© (2006). Bray-Curtis similarity matrices were derived from square-root transformed data. This level of transformation was necessary to down weight for the contribution of rare species (Clarke 1993) (e.g., potential indicators whose occurrence can be negligible) but which may represent an early or unnatural response to nutrient enrichment.

To examine the patterns of variability in the community structure of experimental plots at all locations prior nutrient additions, a mixed-model PERMANOVA was conducted. The plot assigned to the experimental treatment was tested against the mean variation of the three control plots (i.e., T [C1, C2, C3]) as a fixed factor (Underwood 1991, 1994). The effect of location (random) was tested by comparing the community structure to identify if any difference between treatment and control plots varied with

location (Location × Treatment). In addition, the level of random variability among plots was contrasted against the overall error terms for each of the main factors: Treatment (Plot nested in treatment) and Location (Plot nested in Treatment × Location) (Table 2.1)

2.2.5.6. Nutrient effects on macroalgal community

To test the effect of nutrient enrichment on the community structure of treatment plots, the same model prior nutrient additions was undertaken after the nutrient additions. PERMANOVAs were conducted separately for spring and summer, as relevant responses of some functional groups of interest, (e.g., Opportunistic species) are seasonally constrained. The most important taxa in determining community differences as well as the most conspicuous opportunistic species between locations and treatment/controls plots were identified using Similarity Percentage analysis (SIMPER). To test the homogeneity/heterogeneity of the community structure as a signal of disturbance (Warwick & Clarke 1993), the multivariate dispersion between locations was analyzed (PERMDISP). To examine the contribution of functional groups to the dissimilarity patterns among treatments and/or locations a Principal Coordinate Analysis (PCO) based on Bray-Curtis similarities was conducted (Anderson et al. 2008). The contribution of each functional group was calculated using Pearson correlation coefficients and visualized throughout vectors plots on the PCO ordination.

2.2.5.7. Environmental variables

To analyse and model relationships between multivariate biotic data and environmental variables separately for each season, a distance based linear model based on Euclidean distances (DISTLM) was used. Environmental data was normalised to adjust for differences in the scale of the different variables. To estimate the relative distance between the fitted model and the observed data Akaike's information criterion was used (Burnham & Anderson 2002). The ordination of samples described by the species abundance and

the relationship with the abiotic variables were plotted using distance based Redundancy Analysis (Legendre & Anderson 1999). The colinearity of the abiotic data was tested using Draftsman plots, and where high levels of colinearity were detected (e.g., I r I ≥ 0.95) variables were removed from the analysis.

2.2.5.4. Univariate

To test for differences in richness and in the abundance of functional groups between treatment, control and locations, a Mixed-Model ANOVA using the same arrangement of terms and interactions as in the multivariate analysis.
On the other hand, the inter-seasonal variation of nutrient concentrations was tested comparing the three distances from the supply units (10 cm, 1m and >50m) with a two-way ANOVA undertaken between seasons (3 levels) and distances (3 levels) for each location separately. Exposure levels for each plot were analysed between locations with one-way ANOVA. Logarithmic transformations were conducted where analysis of residuals suggested strong linear relationship between the mean and the variance (Quinn 2002). When assumptions were not met with logarithmic transformations, data was Box-Cox transformed (Gotelli & Ellison 2004). If transformations did not reduce variance and skew of data, Kruskal-Wallis and U Mann-Whithney tests were undertaken.

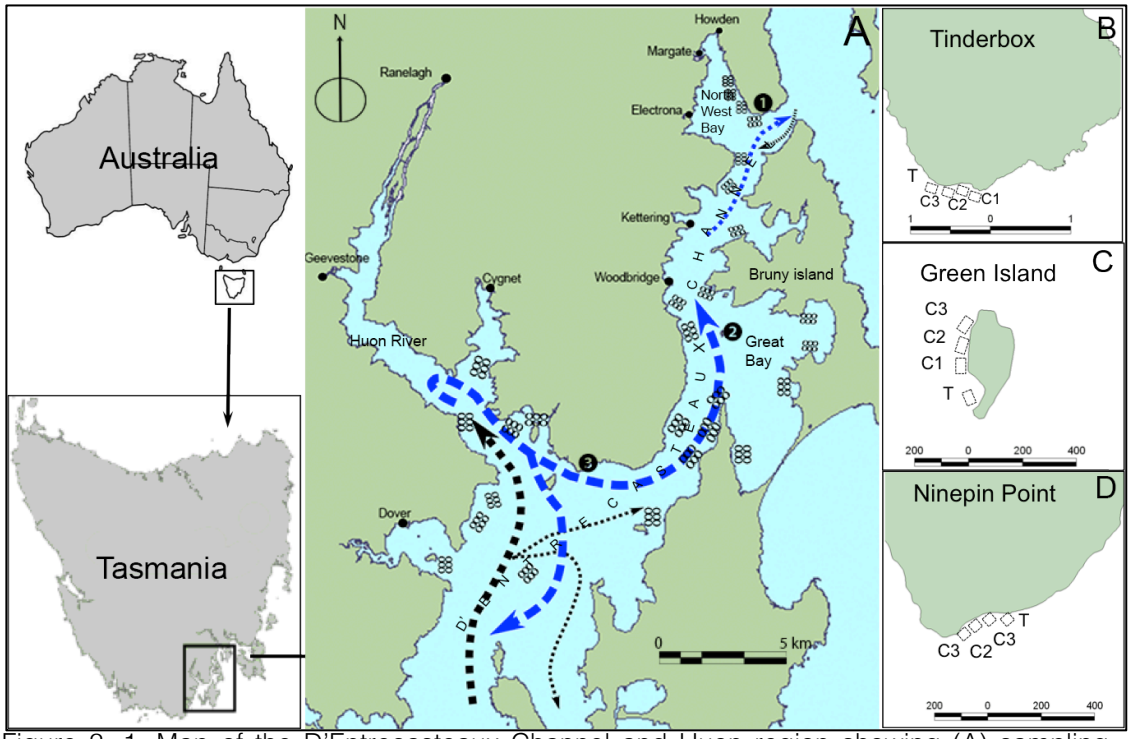

Figure 2. 1. Map of the D'Entrecasteaux Channel and Huon region showing (A) sampling locations (1) Tinderbox, (2) Green Island and (3) Ninepin Pt. B, C and D show deployment of sampling sites within locations. Blue arrows indicate the main pattern of surface circulation; black arrows show bottom circulation. Waste Water Treatment plants (●) are indicated by the site name listed and salmon farms are shown as clustered circles. (Modified of: Ross & Macleod 2010; DPIPWE, 2015).

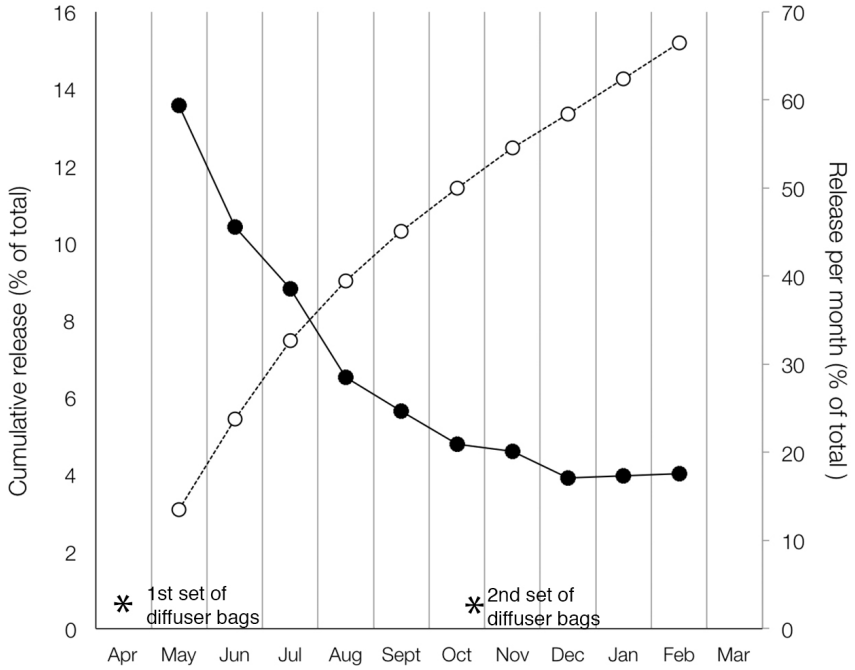

Figure 2. 2. Plot illustrating the theoretical cumulative release rate (clear points) and the monthly release rate (solid points) of the first set of nutrient suppliers used to stimulate nutrient enrichment on macroalgal communities along the D'Entrecasteaux Channel. The nutrient additions were deployed twice during the study period to assure an increase of dissolved nutrient during at least 9 months.

Table 2. 1. Model design to determine the sensitivity of treatment effects between locations

Treatment (Fixed)	Treatment plots vs. Control plots (T vs. C1, C2, C3)
Plot(Treatment) (Random)	Error term for Treatment. It tests if the difference between the Treatment plots and the three Controls stand out from plot to plot variation
Location (Random)	Tests the overall differences among locations
Treatment × Location (Random)	Tests if the Treatment effect (T vs. C1, C2, C3) varied depending on location.
Location × Plot(Treatment) (Random)	Error term for interaction Treatment × Location

Table 2. 2. Easy-to-identify classification on this study based on algal functional groups as defined by general morphology, environmental productivity and susceptibility to disturbance (Steneck and Diether, 1994), and survival strategy (Littler & Littler, 1980). Caulerpales* were classified separately in its own group as late successional form.

(Littler & Littler, 1980)	(Steneck & Diether, 1994)	Sub-classification of functional groups used in this study	Species
Opportunistic form	Fillamentous (Uniseriate)	Opportunistic species	Cladophora, Bangia
	Foliose Algae (single/ multilayered)	Opportunistic species	Ulva spp.
	Corticated fillamentous	Opportunistic species	Polysiphonia
Late successional form	Corticated foliose	Understorey species	Padina, Dyctiota, Zonaria
	Corticated macrophytes	Understorey species	Chondrus, Plocamium
	Leathery macrophytes	Fucales	Sargassum, Phyllosphora
	Leathery macrophytes	Laminariales	Macrocystis, Ecklonia
	Articulated calcareous	Encrusting/ Coralline	Corallina
	Encrusting algae	Encrusting/ Coralline	Lithothamnion
		Caulerpales*	Caulerpa trifaria

2.3. Results

2.3.1. Characterization of reef communities in The D'Entrecasteaux Channel

The community structure of treatment plots prior to the addition of nutrients was not significantly different to control plots, but they were significant differences between locations (Table 2.3). The analysis of functional groups showed that the differences were driven by Understorey species and Encrusting/Coralline algae abundances (Table 2.4). However, the species richness in treatment plots was similar to that of the control plots and between locations ($F = 0.3$; df 2; $p = 0.8$). As per the PERMANOVA analysis, the examination of the multivariate dispersion of the community structure showed significant effects of location (PERMDISP, F 19; df 2; 140; $p < 0.01$). This analysis indicated that the community structure at Tinderbox and Green Island were similar, ($t = 1,7$; P(perm) = 0.09), but that the community structure at Ninepin Point was significantly different with respect to Tinderbox ($t = 4$; P(perm) < 0.01) and Green Island ($t = 5.2$; P(perm) < 0.01). The largest pattern of dispersion was observed at Green Island (Av. centroid deviation 59 ± 1.3 SE), followed by Tinderbox in the upper Channel; (Av. centroid deviation 56 ± 1 SE), while Ninepin Pt. presented the lowest variability between replicate samples (Av. centroid deviation 46 ± 2 SE). This is observed in the principal component ordination analysis (Figure 3), which provided further insight into the algal groups most responsible for location-based differences. Caulerpales appeared to separate the communities of Tinderbox and Green Island, while Fucales, mainly represented by the fucoid Phyllosphora comosa at Ninepin Pt., distinguished the community structure of this location from the other two, where Sargassum fallax was the most abundant fucoid. Encrusting/Coralline algae were key to distinguishing the community composition in Ninepin Pt. Understorey species mostly

represented by *Lenormandia marginata*, and to a lesser extent by *Plocamium angustum*, separated the community of Tinderbox and Green Island in the upper/mid Channel from Ninepin Pt. in the lower Channel.

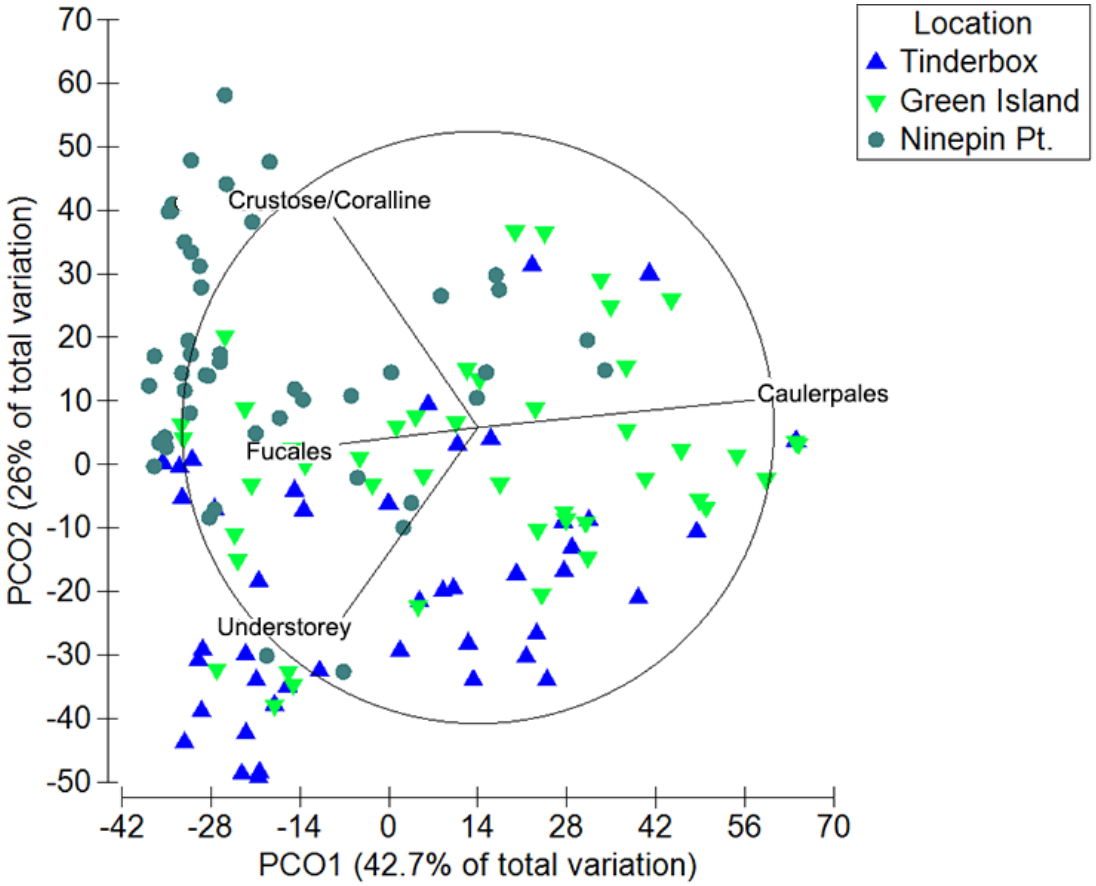

Figure 2. 3. Principal Coordinates Ordination (using Bray-Curtis dissimilarity) of macroalgal community structure (68% of the variation explained) for individual cases from each of the three study locations (n = 140). The vectors show Spearman correlation (r > 0.5) for the functional groups best correlated with the first two axis (Circle indicates a radius of r = 1).

Table 2. 3. Results of Mixed-model PERMANOVA analysis comparing the macroalgal community structure throughout locations during pre-treatment conditions (Autumn).

Source	df	SS	MS	Pseudo-F	P(perm)	Permutations
Treatment	1	9084.5	9084.5	1.1	0.4	9933
Location	2	81318	40659	3.6	0.02	9944
Plot(Treatment)	2	14593	7296.5	0.8	0.6	9927
Treatment × Location	2	18512	9256	0.8	0.5	9933
Location × Plot(Treatment)	4	36084	9020.9	3.3	<0.01	9888
Residuals	131	00003	2706.4			
Total	142	00005				

Table 2. 4. Summary of Mixed-model PERMANOVA comparing macroalgal functional groups throughout locations during pre-treatment conditions (Autumn). (**) Indicates $p < 0.01$, (*) indicates $p < 0.05$.

Functional Group	Treatment		Location		T × L		Plot(Treatment)		Location×Plot (Treatment)	
	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df
Caulerpales	1.1	1	1.9	2	0.93	2	0.74	2	1.7**	4
Understorey	1.1	1	3.3**	2	1.7	2	0.9	2	1.2	4
Opportunistic spp.	1	1	0.9	2	0.7	2	0.5	2	1.3**	4
Laminariales	0.8	1	2	2	0.9	2	0.2	2	0.96	4
Fucales	0.9	1	1.2	2	0.5	2	1	2	2.7**	4
Encrusting/Corallin e	1.2	1	3.1*	2	0.5	2	0.56	2	2.2**	4

2.3.2. Effect of nutrient addition on established macroalgal communities

There was no significant effect of nutrient additions on species richness and community structure during both seasons (Table 2.5). Treatment plots were usually dominated by perennial species (Table 2.6). A similar result was observed with the functional groups (Table 2.6). The nMDS plots suggested higher dispersion in the community structure at the treatment plots (Figure 4 A and C), but these pattern could not being separated from those of controls in all locations (PERMDISP P(perm) > 0.05). Instead, the community ordination suggested location-specific differences in both seasons, where the community at Ninepin Pt. appeared more separated respect the other two. This was confirmed by the multivariate dispersion analysis (PERMDISP P(perm) < 0.01). The communities from upper/mid Channel were strongly represented by Caulerpales, Fucoids and Laminariales. Whilst, in Ninepin Pt. (lower Channel), there were a strong presence of Encrusting/Coralline algae and Fucoids such as *P. comosa*, however, there was also high variability among samples (Table 2.6). Accordingly, no significant differences were observed in any functional group with the exception of Encrusting/Coralline algae, represented by *Lithothamnion* sp.1 (Table 2.6), which showed higher abundance at Ninepin Pt. (Table 2.5 B and D). Opportunistic species appeared to have unusually higher mean abundances in the treatment plot at Green Island during spring (Figure 4 B). However, as a result of the high

level of variability among plots there was no statistically significant response attributable to nutrient additions. During summer there was a broad increase in opportunistic abundances (Figure 4 D), but also no significant differences between treatment and locations. Despite the variability observed during spring, opportunistic species at Green Island were 5-6 times (76%) more abundant in the treatment plot and almost 10 times (125%) more abundant than in plots from other locations. *Polysiphonia decipiens* (Rhodophyta, Ceramiales), Ephyphitic algae and *Ulva* sp. (Chlorophyta, Ulvales) accounted for the 43% of average macro-algae abundance at the treatment plot (Table 2.7). During summer, opportunistic species at Green Island were represented solely by *P. decipiens* (40% average abundance, Table 2.7). In Tinderbox the most abundant opportunistic species in the treatment site were *Chaetomorpha billardierii* and *Polysiphonia decipiens* (20 and 4.5% average abundance respectively; Table 2.7). At Ninepin Pt., only *Ulva* sp. appeared as the most abundant opportunistic species with a 4% of average abundance.

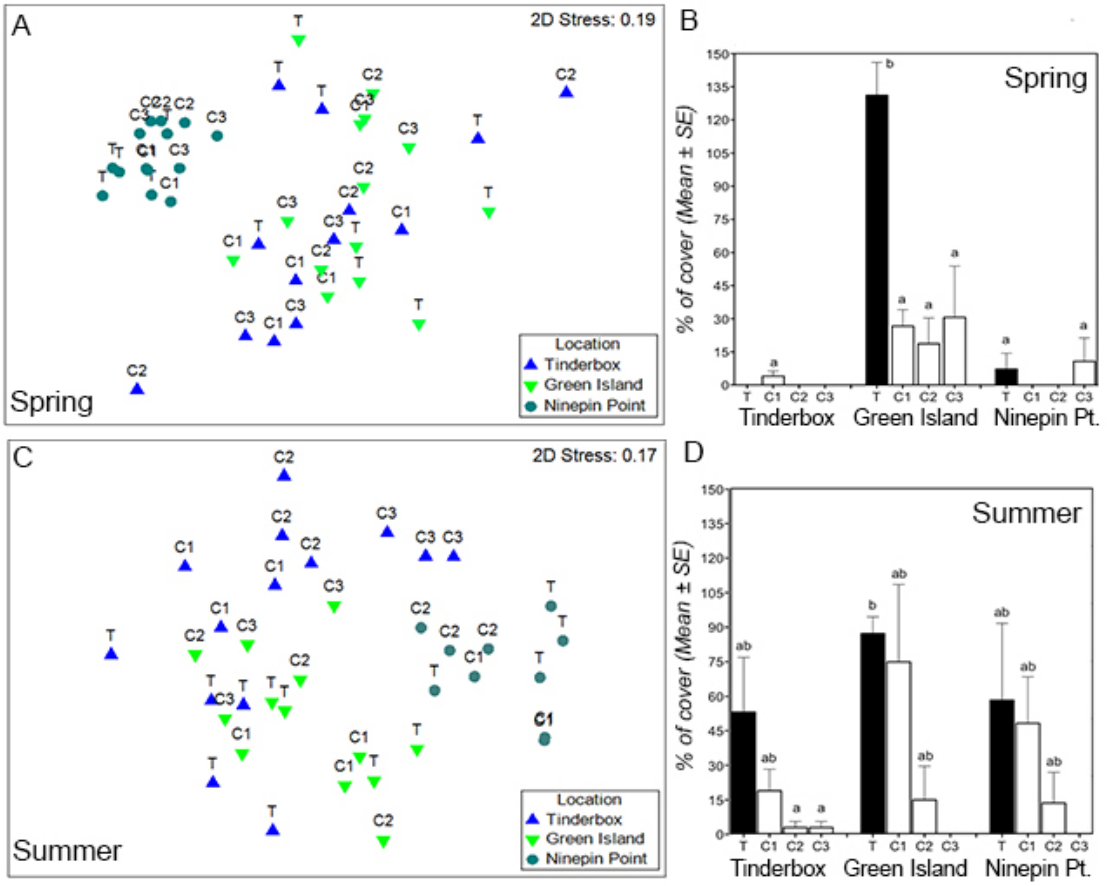

Figure 2. 4. Percentage of cover (Mean ± SE) of opportunistic macro-algae in (A) spring and (B) summer. MDS ordination of individual quadrats based on Bray–Curtis similarities of community structure of three macroalgal reefs during, (C) spring (upper panel) and (D) summer (Lower panel). T = quadrats from treatment plots, C1, C2 and C3 = quadrats from control plots

Table 2. 5. Summary of Mixed-model PERMANOVA on community structure during spring and summer. (**) p < 0.01; (*) p < 0.05)

	Spring		Summer	
Source	df	Pseudo-F	df	Pseudo-F
Treatment	1	1.19	1	1.42
Plot(Treatment)	2	0.52	2	0.76
Location	2	5.89**	2	2.88**
Treatment × Location	2	1.37	2	0.77
Location × Plot(Treatment)	4	1.53*	4	3.2**
Res	29		26	
Total	40		36	

Table 2. 6. Summary of one-way PERMANOVA comparing effect of nutrient additions on macroalgal functional groups and One-way ANOVA for Richness throughout locations during (A) spring and (B) summer. (*) Indicates p < 0.05; (**) Indicates p < 0.01.

A. Spring	Treatment		Location		T × L		Plot(Treatment)		Location×Plot (Treatment)	
Functional Group	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df
Richness	1.5	1	29	2	0.3	2	1.1	2	1.7	4
Caulerpales	0.5	1	5.8	2	1.6	2	1.1	2	0.9	4
Understorey	7.5	1	1.5	2	0.7	2	0.3	2	2	4
Opportunistic spp.	0.5	1	7.9	2	4.7	2	8.1*	2	1.4	4
Laminariales	0.4	1	1.2	2	3.3	2	0.9	2	0.07	4
Fucales	0.01	1	6.8	2	2.2	2	0.4	2	0.9	4
Crustose/Coralline	0.5	1	80*	2	1.1	2	0.6	2	0.7	4
B. Summer	Treatment		Location		T × L		Plot(Treatment)		Location×Plot (Treatment)	
Functional Group	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df
Richness	0.3	1	0.4	2	0.7	2	0.1	2	1.2	4
Caulerpales	1.1	1	1.3	2	4.5	2	0.9	2	0.7	4
Understorey	0.5	1	7.9	2	0.6	2	0.3	2	0.9	4
Opportunistic spp.	1.6	1	7.9	2	0.7	2	11*	2	0.6	4
Laminariales	0.4	1	1.2	2	6.5	2	2.7	2	1.4	4
Fucales	0.05	1	2.2	2	1.5	2	0.8	2	0.8	4
Crustose/Coralline	0.5	1	52*	2	0.07	2	0.8	2	4*	4

Table 2. 7. Summary of SIMPER analysis identifying most abundant macroalgal taxa (Contributions > 1%) to the average Bray-Curtis dissimilarity between treatment and control sites across locations during spring and summer. Average abundance, ((δ_i/SD(δ)) Standard deviation of individual dissimilarities, (C%) Individual contribution, (Cum%) cumulative percentage on individual contributions

SPRING					SUMMER				
TINDERBOX									
Treatment; Av. similarity 33					Treatment; Av. similarity 40				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%	Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Sargassum Fallax	5.59	0.9	43.42	43.42	Ecklonia radiata	7.39	1	36.12	36.12
Caulerpa geminata	5.79	0.9	41.19	84.61	Caulerpa simpliscula	4.28	1	21.54	57.66
Caulerpa trifaria	3.12	0.4	9.92	94.53	C. billardierii	3.81	1	19.7	77.36
					Caulerpa longifolia	3.92	0.3	7.43	84.79
					Polysiphonia decipiens	2.65	0.3	4.54	89.33
					Sargassum Fallax	2.66	0.3	3.56	92.89
Control; Av. similarity 34					Control; Av. similarity 33				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%	Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%
Ecklonia radiata	5.3	1	34.6	34.6	Acrocarpia paniculata	5.4	0.6	24.67	24.7
Lenormandia marginata	3.3	0.8	17.7	52.3	Lenormandia marginata	3	1	22.95	47.6
Caulerpa trifaria	2	0.4	11	63.7	Sargassum Fallax	4.2	0.6	18.85	66.4
Plocamium angustum	2.6	0.5	10.6	74.4	Caulerpa longifolia	3.2	0.4	9.22	75.7
Sargassum Fallax	2.5	0.4	7.8	82.2	Lithothamnion sp. 2	2	0.3	6.18	81.8
Caulerpa geminata	2.3	0.4	7.6	89.7	Red recruits	1.6	0.4	5.67	87.5
Cirrulicarpus polycoides	1.2	0.4	5	94.7	Caulerpa geminata	1.8	0.3	3.81	91.3
GREEN ISLAND									
Treatment; Av. similarity 32					Treatment; Av. similarity 48				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%	Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%
Red algae (recruits)	4	1	23.4	23.4	P. decipiens	9.3	19.8	40	40
Polysiphonia decipiens	4.7	0.6	17.2	40.6	Caulerpa simpliscula	6.7	0.9	18.9	58.9
Epiphytic load	4	0.6	13.7	54.3	Ecklonia radiata	4.7	0.9	9.2	68.2
Ulva sp.	3.8	0.6	12.4	66.7	Hemineura frondosa	3	0.9	9	77.3
Caulerpa remotifolia	3	0.6	11.5	78.3	Plocamium sp.	2.9	0.9	7.2	84.5
Caulerpa geminata	2.8	0.3	5.7	84	Caulerpa trifaria	4	0.4	5.4	89.9
Ecklonia radiata	3.4	0.3	5.4	895	Sargassum Fallax	3.3	0.4	3.8	93.8
Caulerpa simpliscula	2.8	0.3	4.3	93.8					
Control; Av. similarity 350.3					Control; Av. similarity 31				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%	Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%
Caulerpa simpliscula	5.6	1	24	24	Sargassum Fallax	4.4	0.7	29.7	29.7
Ecklonia radiata	4.9	0.6	16	40.6	Caulerpa simpliscula	3.9	0.7	18.4	48
Sargassum Fallax	3.9	1	15.7	56	Ecklonia radiata	4	0.5	14.6	62.7
Caulerpa trifaria	3.9	0.7	15	71.6	Caulerpa trifaria	3.3	0.5	9.3	72
Ulva sp.	2.2	0.6	6.6	78	Polysiphonia decipiens	3.2	0.4	8.5	80.6
Red algae (recruits)	2.5	0.6	6.4	84	Caulerpa brownii	2.7	0.5	7.73	88.3
Gelidium australe	2	0.6	5.6	90	Caulerpa geminata	2.4	0.2	4.8	93.1
NINEPIN POINT									
Treatment; Av. similarity 59					Treatment; Av. similarity 49				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%	Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%
Lithothamnion sp. 1	9	7.8	42	42	Lithothamnion sp. 2	7.02	2.2	44.89	44.89
Phyllosphora comosa	6.4	1	19.8	62	Lithothamnion sp. 1	7.48	3	44.85	89.74
Zonaria sp.	5.2	1	17.2	79.2	Acrocarpia paniculata	3.66	0.4	6	95.74
Lithothamnion sp.2	3.5	1	11.3	90.5					
Control; Av. similarity 54					Control; Av. similarity: 52				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%	Species	Av.Abund.	δ/SD(δ)	C(%)	Cum.%
Lithothamnion sp. 1	7.3	6	37	37	Lithothamnion sp. 1	7	4	25.8	25.8
Sargassum Fallax	4.3	3	20.5	57.6	Caulerpa geminata	5.7	1.3	17.3	43
Phyllosphora comosa	5.5	0.8	16.1	73.8	Phyllosphora comosa	6.7	0.8	16	59
Zonaria spp.	3.2	1	10.7	84.5	Acrocarpia paniculata	5.9	0.7	14	74
Acrocarpia paniculata	3.4	0.55	6.8	91.3	Lithothamnion sp. 2	5	0.7	11.6	85.8
					Sargassum Fallax	3.15	0.5	4	89.7
					Ulva sp.	2	0.8	3.9	93.6

2.3.2. Environmental variables

2.3.2.1. Wave exposure

Each of the study locations differed in the level of wave exposure, as determined by the openness index (One-way ANOVA; F 4.6, df 2, p = 0.04). The plots in Tinderbox in the upper Channel showed the lowest wave exposure (Mean 7.2^{-6} ± 4.2^{-7} SE), as this location is geographically situated at the east end of North West Bay, which offers more protection of wind and swells from outer Channel. Green Island, in the mid Channel, was relatively more exposed to wind-driven waves, therefore, the sampling plots were found to have an intermediate wave climate (Mean 7.7^{-6} ± 7.6^{-7} SE) showing higher openness values than Tinderbox, but lower than Ninepin Pt. (lower Channel). Ninepin Pt. is geographically more exposed to winds from the southern end of the Channel and to swells from the southern ocean, and as a consequence had the highest openness values (Mean 9.6^{-6} ± 5.3^{-7} SE).

2.3.2.2. Salinity and Temperature

Surface salinity (~1m depth) varied significantly among locations (Figure 2.7 A). During spring (six months post enrichment) salinity levels peaked in plots at Green Island (31 PSU), while the lowest levels were observed at Tinderbox and Ninepin Pt. (~ 21–23 PSU, respectively). Surface water temperatures were similar at all locations, and tended to reflect seasonal expectations, with minimal values in winter (10 C^{o}) and maximum levels in summer (18C^{o}). In spring (October) average temperatures at Green Island were 2 C^{o} higher than at Ninepin pt. in the lower Channel area.

2.3.2.3. Nutrient water column

The addition of nutrients significantly increased measured concentrations in the water; however, there was pronounced spatial and temporal variability at medium (Location) and small-scale (plot). Dissolved inorganic nitrogen (i.e., NH3 + NOx) increased significantly in the immediate vicinity of the nutrient source at 10 cm excepting at Ninepin Pt. in spring (Table 8, Figure 2.5 A). However, elevated nutrient concentrations were not detected at 1m at any location, although average water column levels at this distance were still higher than ambient concentrations, as shown by the points above the 1:1 line (Figure 2.5 B). A comparable pattern was observed with the fraction of reactive phosphorus in treatment plots (Figure 2.6 A), which similarly showed significant increases in the vicinity of the source but decreased at 1m. Values particularly at Tinderbox during spring were similar and even frequently below ambient concentrations (Figure 6 B). Whilst concentrations of DIN at Green Island and Ninepin Pt. were typically higher at 1m relative to ambient concentrations during both summer and spring the results were highly variable on a local scale (i.e. within site). The difference in the degree of enrichment, i.e. the level to which DIN concentrations increased relative to the ambient values, varied among locations (Figure 2.5 and 2.6 insets). At all locations DIN concentrations were more than 5 μM/ l^{-1} higher 10 cm from nutrient supply units than at the ambient "control" sites (and up to 20 μM/ l^{-1} in Ninepin Pt. during winter). However, at 1m this difference was generally less than 1.4 μM/l^{-1}, with the greatest divergence being at Green Island at 1m in spring (Figure 2.5 B insets). There was little difference in FRP between enriched and control conditions at either distance. Maximum differences were observed at 10 cm in Ninepin Pt. while at 1m these differences were minimal they were greater and consistent at Green Island. Maximum average

concentrations (DIN = 13 $\mu M/l^{-1}$, FRP = 2.3 $\mu M/l^{-1}$) were observed at Ninepin Pt., which were higher than Tinderbox (DIN = 7.8 $\mu M/l^{-1}$, FRP = 1.5 $\mu M/l^{-1}$) and Green Island (DIN= 4.7 $\mu M/l^{-1}$, FRP = 1.2 $\mu M/l^{-1}$).

2.3.3. Relationship between environmental variables and community structure

Environmental factors helped explain the separation of community structures between locations (DISTLM Marginal Test, $p < 0.05$). The first two axes of the dbRDA explained 83.5% of the fitted variability and 19% of the overall variability prior nutrient additions (Figure 2.7 A). Temperature and salinity (32% of the community variation) appear to be significant factors separating the macroalgal assemblages at Ninepin Point from the assemblages in Green Island. While phosphorus and dissolved inorganic nitrogen (DIN), and to a lesser extent wave exposure (Openness) separated the communities of Tinderbox and Green Island. Openness explains 13% of the overall variation, while DIN and phosphorus explained 11 and 10 % respectively. After nutrient additions the community structure was clearly related with some key environmental factors (DISTLM Marginal Test, $p < 0.05$). The first two axes explained 81.8% of the fitted variability in spring (Figure 2.7 A) and 74.5 % of the variation in summer (Figure 2.7 B), with the overall variability between 37.5% and 29.8% respectively. Light and wave exposure (Openness) differentiated the communities at Ninepin Pt. from Tinderbox/ Green Island after six and nine months post-enrichment, suggesting that these factors may be key drivers in the differentiation of macroalgal assemblages at the lower Channel location (Figure 2.7 B and C). These environmental variables correlated significantly with the community variations during both seasons (Marginal Test, $p < 0.05$). On the other hand, salinity, and to a lesser extent phosphorus and temperature appeared to be important factors in distinguishing the communities in spring. In summer, the separation of

Tinderbox and Green Island communities was underpinned by variations in salinity and DIN (Figure 2.7 C). Interestingly, the treatment sites appeared similar in spring but during summer the distribution of treatment plots was more closely associated with DIN, as the main explanatory variable (Figure 2.7 C). Although the treatment effect was not statistically significant, due to high intra-plot variability, it is possible that the relative increase in abundances of opportunists in particular for Green Island (as suggested in figure 2.4) could still be influencing the community structure as shown by the wide separation of the communities from treatment plots (Figure 2.7 B and C). This may suggest that effects of nutrient additions and the associated environmental differences (e.g., Openness, DIN, light) may have promoted a level of impact/ response on this location.

Table 2. 8. Summary of two-way ANOVA and non-parametric one-way Kruskal-Wallis on dissolved inorganic nutrients per location between seasons and distance from supply units (10 cm, 1 m, >50 m). Table shows only significant interactions for each nutrient analysed.

	Nutrient	df	F	p-value
A. Tinderbox	NH_3 (Season × Distance)	4	2.6	0.05
	FRP (Distance)	2	41.7	p<0.01
	NOx (Season × Distance)	4	8.5	p<0.01
B. Green Island	NH_3 (Distance)	2	14.1	p<0.01
	FRP (Season)	2	---	p<0.01
	FRP (Distance)	2	---	p<0.01
	NOx (Season × Distance)	4	14.1	p<0.01
C. Ninepin Pt.	NH_3 (Season × Distance)	4	12.2	0
	FRP (Season)	2	---	0.01
	FRP (Distance)	2	---	0.03
	NOx (Season × Distance)	4	12.2	p<0.01

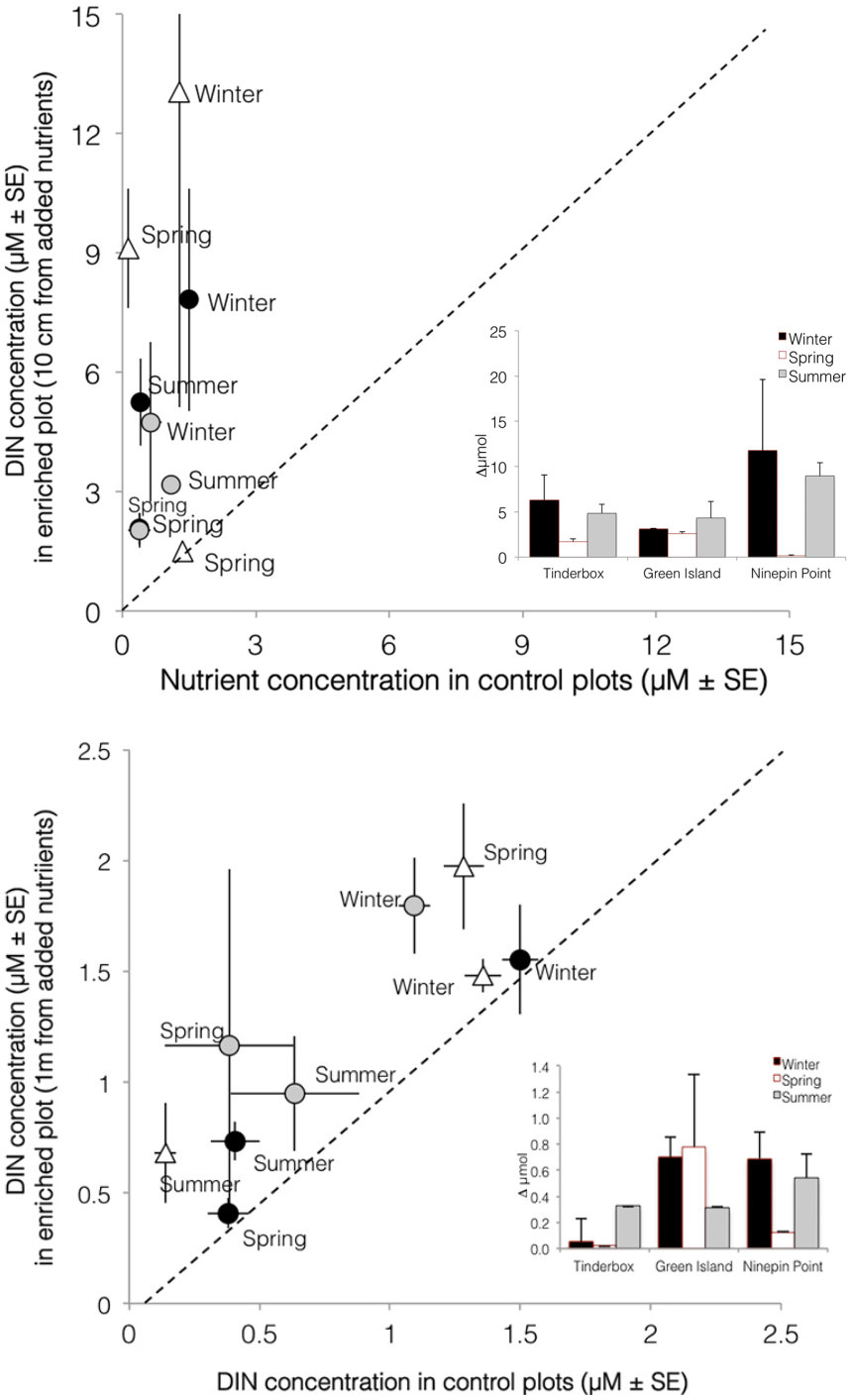

Figure 2. 5. Scatter plot of DIN concentrations (NH3+NOx) in the enrichment treatments (10 cm A; 1m B from added nutrients) vs. average DIN concentration at control plots for all sites and seasons. Dashed line represents the 1:1 line. Black circles = Tinderbox; Grey circles = Green Island; Triangles = Ninepin Pt. The insets show the frequency of the Δ mean DIN concentration (±SE), the difference between mean concentrations in the enriched treatment (10 cm and 1m from added nutrients) and the corresponding controls, for all sites and seasons.

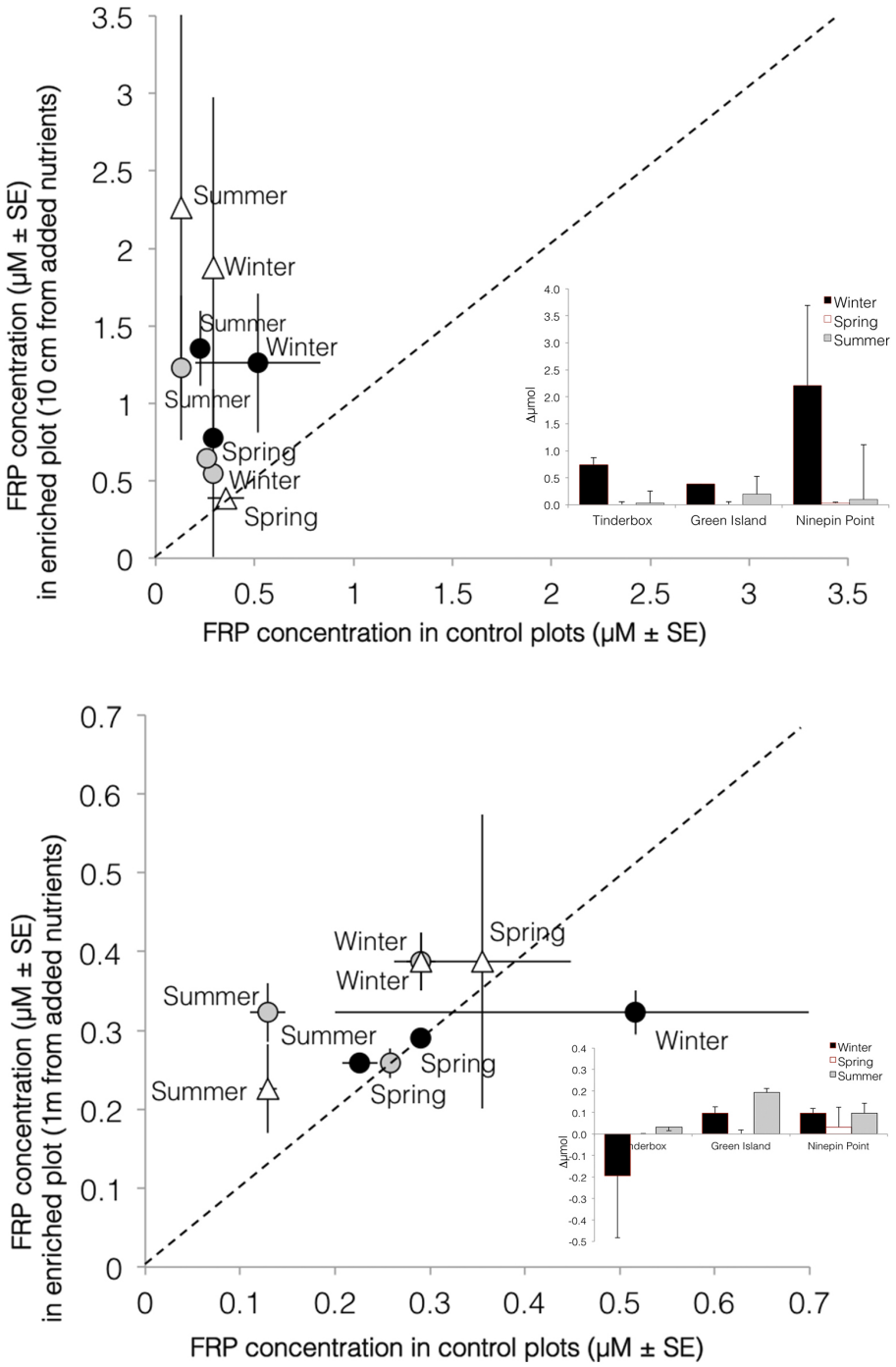

Figure 2. 6. Scatter plot of fraction of reactive phosphorus (FRP) concentrations in the enrichment treatments (10 cm A; 1m B from added nutrients) vs. average DIN concentration at control plots for all sites and seasons. Dashed line represents the 1:1 line. Black circles = Tinderbox; Grey circles = Green Island; Triangles = Ninepin Pt. The insets show the frequency of the Δ mean DIN concentration (±SE), the difference between mean concentrations in the enriched treatment (10 cm and 1m from added nutrients) and the corresponding controls, for all sites and seasons.

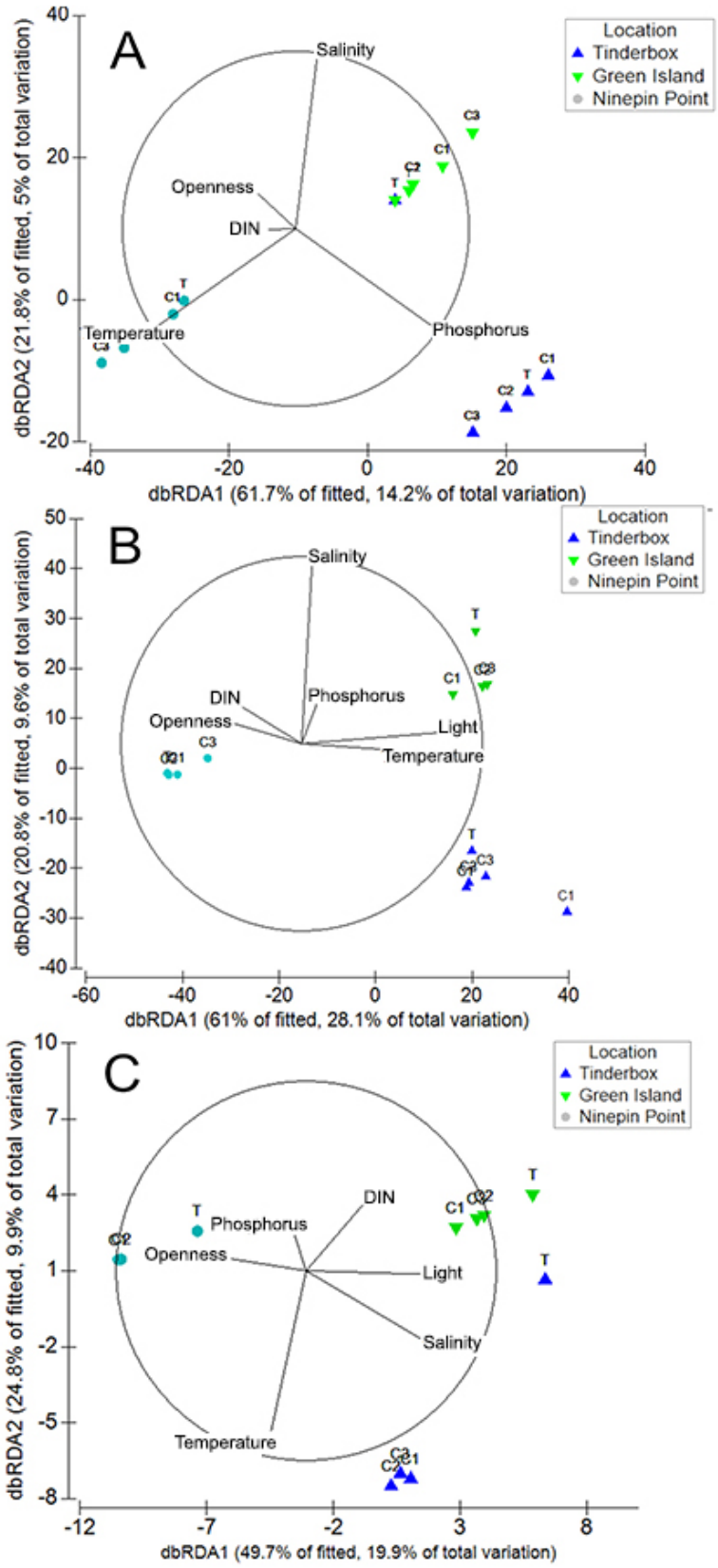

Figure 2. 7. Distance-based RDA ordination relating abiotic factors and community composition showing (A) pre-treatment conditions (n= 140) (B) after six months (n = 41) and (C) after nine months (n = 37) and of exposure to a slow-release fertilisers. T = treatment sites, C1, C2, C3 = Control sites.

2.4 Discussion

The results show a measurable nutrient increase in the water column (between 0.5 to 1.4 µmol l^{-1}) at 1m from the nutrient sources relative to ambient values, and elevated values exceeded by at least one order of magnitude (> 10 µmol l^{-1}) ambient values at 10 cm from supply units. However, this had no substantial effect on assemblages of opportunistic species on treatment plots. There was a pronounced decline in nutrient concentrations observed 1 m from the supply units.

Experimental work in laboratory has shown that in order to stimulate a biological response nutrient concentrations must exceed thresholds values (Fujita & Goldman 1985, Fujita et al. 1989). However, this response may differ under field conditions. Previous studies have suggested that relatively pristine ecosystems or environments subjected to low nutrient conditions may show no effects in the short term (See Ruiz et al. 2001, Karez et al. 2004, Fourqurean et al. 2010). In this scenario biophysical factors other than nutrient availability seem to play critical roles in the control of opportunists on macroalgal reefs (Bokn et al. 2002, Bokn et al. 2003, Kraufvelin et al. 2006, Kraufvelin et al. 2010). In this experiment, the treatment effect was probably strongly affected by local hydrodynamic diffusion, which was marked and appears to have created a steep spatial gradient in nutrient concentrations.

The local differences in the wave climate and light seemed to play a significant role in defining the macroalgal assemblage. In this study the spatial differences in wave exposure and community composition were comparable to those reported by Barrett (2001) and Oh (2015) for similar locations within this estuarine system. Wave exposure seems to explain the predominant community composition in the upper/middle Channel area characterised by Caulerpales, understorey species, *Sargassum fallax* and a

higher abundance of opportunistic species. This differed from the more exposed sites at Ninepin Pt. where the community was characterised by a Canopy-encrusting algae association – i.e., *Phyllosphora comosa* and *Lithothamnion* sp. – and a low occurrence of opportunistic species (Edgar 1983b, Edgar 1984, Sanderson & Thomas 1987, Barrett et al. 2001, Oh et al. 2015). Wave exposure is a major structuring factor for habitat composition in rocky reefs (Connell 1972, Eckman et al. 1989a, Eckman et al. 1989b, Menge et al. 1993, Wernberg & Connell 2008). Accordingly, wave exposure has shown to explain community composition and the frequency of structuring species in southern Australia (Wernberg & Connell 2008) and Tasmania. (Hill et al. 2010). Hence, it may be possible that gradients in habitat structure indicated by wave exposure may also reflect the probability of occurrence of natural constraints for opportunistic algae and nutrient enrichment.

For instance, shading (Bokn et al. 2003, Kraufvelin et al. 2006, Eriksson et al. 2007) and whiplash effect exerted by large brown algae (Kiirikki 1996, Wernberg & Connell 2008) such as *P. comosa*, as well as greater biomass export of ephemeral algae by waves (Pihl et al. 1999, Denny 2006) may have a significant influence on opportunistic proliferation and nutrient diffusion at Ninepin Pt. sites. Similarly, limitation in substrate availability driven by greater frequency of Caulerpales and encrusting algae (Connell 2003), especially at Tinderbox (upper Channel) and Ninepin Pt. (Lower Channel), may have limited the propagation of opportunistic algae. Wave exposure may be a useful tool for a rapid evaluation of environmental susceptibility, since macroalgal distribution may provide an environmental context to test such a premise (Wernberg & Connell 2008). At this point, the challenge is to incorporate the understanding of the relationship between the biological information that wave exposure and other forcing factors may provide into a

criterion that provides a range of susceptibility to nutrient enrichment in estuarine habitats.

Spatial differences in light attenuation driven by coloured organic matter transported by the Huon River, particularly at the more exposed locations in Ninepin Pt. can cause a significant change in the light environment (Butler 2006) and consequently in the structure of macroalgal reefs (Reed & Foster 1984, Barrett et al. 2009). These natural features certainly can influence effects of nutrient enrichment (Fong et al. 1996, Kamer & Fong 2000, Lotze & Schramm 2000, Sousa et al. 2007). Consequently, the combined effect of light attenuation and wave exposure may indicate local mitigation mechanisms, offsetting the deleterious effects of nutrient enrichment in areas such as Ninepin Pt. and thereby enhancing the comprehension of an environmental context to infer protection/ resilience in these reef systems.

The relationship between salinity, temperature, DIN, phosphorus and community structure in the upper and mid Channel indicate that these factors may influence the responses to nutrients differently – this was particularly evident at mid the Channel location (Green Island) which experimented the highest abundance of opportunistic species. This location seemed to respond in a different way given an intermediate community structure, an intermediate exposure to wave and probably a different water mix given its intermediate location in the Channel away from major fresh water inputs in the upper and lower Channel. Freshwater inputs have shown to exert not only a strong influence on salinity, temperature and light attenuation but in growth and persistence of opportunistic macroalgae in estuarine systems (Fong et al. 1996, Kamer & Fong 2000). The influence and the size of freshwater subsidies has been considered as major nutrient source in this system (Ross & Macleod 2013). However, the geographic distribution, range of action and

potential effects of local drains on the associated reef response may be crucial considering the major effect on controlling variables directly related with the response of bloom-forming algae to nutrient enrichment.

The physical variations along The D'Entrecasteaux Channel illustrate the complex biophysical gradient that interacts with anthropogenic nutrients. In turn, this emphasizes that geographical differences may encompasses a particular biological responses in this ecosystem Therefore, the variability of opportunistic proliferation during spring and summer may simply be a function of physically induced nutrient advection and diffusion on uptake rates at each location. However, being able to typify the underlying characteristics that define the systems capacity to assimilate nutrients could be very useful in identifying spatial differences in particular areas resilience/ sensitivity. Similar situations, i.e. that some systems may cope better than others, have previously been documented on the response to the impacts of finfish aquaculture in soft-bottom habitats in southeast Tasmania (Macleod et al. 2006, Macleod et al. 2007).

Environmental variability may modify the response of macroalgae to a given nutrient enrichment pressure (Kraufvelin et al. 2006). This leads to complex patterns far from the conventional cause-effect response observed in small-scale experiments (Pedersen & Borum 1997, Kraufvelin et al. 2002, Karez et al. 2004). The current results reflect the natural variability of shallow reefs in SE Tasmania and so are relevant for coastal planning in this region. Under these conditions a substantial pulse of nutrient enrichment (i.e. equivalent to the load associated with the nutrient outputs of a small Atlantic salmon culturing operation - 80 kg of N; 64 kg P, and 8 kg of Fe) was not effective to stimulate a significant community change. The nutrients supplied (i.e. 6 to 9 μmol l^{-1} DIN) largely reached optimal range for growth of fast-growing

species of laboratory experiments (e.g., 1-10 µmol l^{-1} DIN; Fujita 1985, Fujita et al. 1989, Pedersen & Borum 1997), but were substantially reduced at 1 m from the supply units. Several studies using short-term pulses of nutrient enrichment have shown similar effect attributed to physical and biological variability (Pihl et al. 1999, Bokn et al. 2003, Kraufvelin et al. 2006, Painting et al. 2007, Kraufvelin et al. 2010) or the relatively pristine nutrient status of the system (Ruiz et al. 2001, Fourqurean et al. 2010). These results suggest that major changes in assemblages of opportunistic species may occur late in the process of eutrophication (Schramm 1999, Cambridge et al. 2007). However, placing the attention on those circumstances where nutrient loads exceed threshold concentrations is a highly reactive strategy, as critical changes in nutrient conditions preceding macroalgal blooms may occur rapidly and as such it may be difficult to predict and respond to these in a timely fashion (Cambridge et al. 2007), and a more proactive approach may be desirable. This study provides a more realistic (accurate) assessment of potential responses because it takes into account the combined effects of large-scale environmental variation found under real world conditions (Wernberg et al. 2012).

2.4.1. Implication for spatial management

From this study, it is possible to infer that reefs habitats may respond differently in the upper/mid portion of the Channel compared with more exposed sites in the lower area. Thus, the understanding and the prediction of the likely consequences of nutrient enrichment are critical for management in the D'Entrecasteaux Channel. However, this study found that natural variability in a range of biological and physical drivers has the potential to affect early responses of opportunists. Where the environmental conditions are appropriate (i.e. where exposure gradients are higher, water flow is good

and light availability is limited) opportunistic species do not alter the structure and function of the community, in fact they may actually act as a mechanism to offset nutrient fertilisation by removing nutrients from the system.

In this study, the quantification of the response of the assemblage of opportunistic species of macroalgae did not provide a clear indication of nutrient enhancements. However, it could provide us with an idea of system capabilities that may modify the response to nutrient enrichment. Consequently, the ability to detect a significant effect may be because there was no significant effect to detect, or it may also have been because the experimental design was compromised by 1) natural resilience to nutrient enrichment, i.e. nutrients were rapidly absorbed/diluted and their effects attenuated in the surrounding environment 2) a short-term enrichment regime and 3) insufficient replication levels to separate the observed response from small-scale natural variability. Due to the high cost of monitoring reef communities, subtler indicators of change, for example, community succession and physiological traits of macroalgae may be able to indicate small gradual variations in nutrient regimes. Overall, the results show that at a regional scale, the determination of abiotic driven variation of community characteristics, and predictions based on indices of these environmental drivers may help in understanding the spatial and temporal extent of variation in nutrient-related effects (de Jonge 2007, Painting et al. 2007, Juanes et al. 2008).

2.4.2. Conclusion

The enrichment pulse in this experiment did not have the intensity to produce a major disturbance on the balance of the community. That said this might be the condition of many coastal systems subjected to moderate nutrient loads. However, although optimistic, an absence of proliferation of bloom-forming

algae may not necessarily indicate a healthy ecosystem status. Instead, abundance of opportunistic species may not be the best predictor of community impacts on relatively pristine systems in a gradual process of enrichment. All biological responses are a function of a suite of prevailing biophysical factors (such as hydrodynamics and light or community composition), and the combined measurement of biophysical factors may be more informative of the systems potential sensitivity or resilience to impacts. For instance, nutrient effects may be more frequently observed at moderate wave-exposure (Oh et al. 2015).

More importantly for interpretation of the experimental results presented here, is the understanding of how the physical drivers of such differences (current patterns, wave exposure, etc.) varied spatially, and how these physical drivers interact with biological factors, which may, in turn, influence the availability and use of exogenous nutrients. The marine eutrophication process is clearly complex and further understanding of multiple environmental mechanisms that would modulate the probability in the occurrence of bloom-forming species is required for effective prevention and amelioration of human-induced change in coastal habitats.

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3. Chapter 3. Effects of nutrient enrichment on the spatial variability of macroalgal succession, a “natural” experiment

3.1. Introduction

Temperate reefs typically have a high level of biodiversity and endemism, and support assemblages of both economically and ecologically important invertebrates, fish (Tegner & Dayton 2000, Barrett et al. 2009) and macroalgae (Rebours et al. 2014). Shallow coastal reefs can mitigate deleterious effects of global climate change on the coastal systems, reducing water flow and wave action enhancing sediment deposition, and consequently, carbon sequestration (McLeod et al. 2011, Duarte et al. 2013b). Macro-algae communities are fundamental for the structure of these important benthic habitats, and as a consequence ensuring normal patterns of recruitment and succession is critical to maintain local biodiversity (Reed 1990, Santelices 1990, Vadas et al. 1990) and ecosystem services. However, there is a lack of information on recruitment processes, with most data coming from studies targeting individual canopy-forming species, such as *Ecklonia radiata* (Wernberg 2009, Wernberg & Vanderklift 2010) and *Sargassum* spp. (Kendrick & Walker 1994, Kendrick et al. 1999). Only a few studies (See Lotze et al. 2001, Valentine & Johnson 2003, Toohey et al. 2007 and references therein) address the recruitment and early development of complete assemblages on subtidal reefs. These studies showed that recruitment, capacity of recovery and benthic community composition in subtidal communities are mainly mediated by algal canopy, top-down (i.e, grazing) and bottom-up (i.e., nutrient loading) processess during microscopic stages. Consequently, the underlying processes that support species turnover, interactions with environmental factors and the effect of

nutrient enrichment during recruitment and succession are still poorly understood (Smale et al. 2011). Alterations to the bottom-up processes (i.e. nutrient loading driving primary productivity) caused by anthropogenic nutrients and the effect on early patterns of community organization may have significant effects on the adult community. These effects may differ depending on the initial community structure. Consequently, understanding the spatial variability of both mature and successional macroalgal communities is important in understanding not only how the system functions as a whole but also how it might respond to the impact of coastal eutrophication.

Coastal habitats are subjected to multiple biophysical and human stressors, which interact in complex and unpredictable ways, leading to widespread alterations in community structure, biodiversity and ecosystem services (Wernberg et al. 2011). Coastal eutrophication can have a significant impact on these environments: it may favour the rapid colonisation of fast-growing species, which have been shown to monopolise the substrate and compete for nutrients and light with the resident habitat-forming species (Raberg et al. 2005, Kraufvelin 2007). Macro-algae community development has been shown to be highly sensitive to nutrient enrichment (Lotze et al. 2000, 2001, Worm et al. 2001, Worm & Lotze 2006a), but community succession is also highly susceptible to changes in environmental conditions more broadly (Santelices 1990). The environmental conditions associated with coastal reefs are highly complex and variable and as such the ability to discriminate a direct relationship between nutrient loads and changes in marine vegetation is very challenging (Krause-Jensen et al. 2008). A number of local factors other than elevated nutrient concentrations have been shown to influence community response (Duarte et al. 2013a).

For instance, studies have shown that water flow (Martins et al. 2001, Kraufvelin 2007, Kraufvelin et al. 2010), light (Hillebrand 2005), as well as, temperature and salinity can influence growth and abundance of bloom-forming macro-algae (Fong et al. 1996, Kamer & Fong 2000). However, community properties such as diversity, the presence of canopy-forming species (Eriksson & Bergstrom 2005, Eriksson et al. 2006a, Eriksson et al. 2007), grazing and predation (Duffy & Hay 1991, Hauxwell et al. 1998 (Russell & Connell 2005, 2007) can counteract the overgrowth of bloom-forming species. For example the synergistic effects of light limitation from canopy-forming species in sites subjected to greater water motion and varying salinity gradients, may enhance the grazing effect on fast-growing species (Krause-Jensen et al. 2007b). This inability to accurately predict the impacts of nutrient enrichment against a dynamic background nutrient regime has been a key factor in undermining the development of suitable management practices (Krause-Jensen et al. 2007b).

Understanding the spatial distribution at which these differences take place may provide insights to clarify the spatial susceptibility of reefs to nutrient enrichment. This needs experiments to be undertaken at a scale that better reflects these interactions under "real world" conditions are critical for a realistic characterisation of the response of benthic communities. This will also help identify meaningful and location-specific biological indicators of eutrophication; something that has been recognized as essential for opportune management in coastal areas (Bokn et al. 2002, Bokn et al. 2003, Kraufvelin et al. 2006, Gorman et al. 2009).

Currently, 39% of the world's population lives in coastal areas and this number is expected to increase markedly over the next few decades (Curran et al. 2002). Therefore, anthropogenic pressures on coastal margins are

growing, particularly with significant increases in urbanisation, associated industries, agricultural and aquaculture practices. The synergistic effects of global climate change will escalate the potential for negative effects on the structure of coastal communities (Lotze & Worm 2002, Douvere 2008, Wernberg et al. 2011, Duarte et al. 2013a, Duarte et al. 2013b). Therefore, the understanding of the combined effect of broad-scale environmental variations on nutrient enrichment and early macroalgal community development and identifying indicators, which can be used to predict the potential for catastrophic change well in advance, remains a key knowledge gap.

Understanding how any particular ecosystem might be susceptible to nutrient fertilization will depend on characterising the biophysical attributes for that particular system and identifying how nutrient enhancement interacts with those factors to produce an adverse response. This study sought to determine whether: 1) early development stages of macroalgal communities were particularly sensitive to *in situ* nutrient additions; 2) spatial variations in environmental factors (nutrients, light, salinity, temperature and wave exposure) can regulate the response of the community development and; 3) how the resultant information might help management and monitoring efforts.

3.2. Methods

3.2.1. Area of study

The D'Entrecasteaux Channel and Huon Estuary region is situated on the western side of Bruny Island and southeast Tasmania and forms an expansive and interconnected estuarine system. Fringing reefs and low profile reef composed principally by limestone provide reliable habitat conditions for seaweeds settlement throughout the Channel (Barrett et al. 2001). These habitats represent 23 % of the coastline in this region and present a structurally complex association of macro-algae. Previous studies have shown that this system has extensive cover of *Ecklonia radiata* (a low canopy-former) and *Acrocarpia paniculata* which is frequently interspersed by fucoids such as *Sargassum* spp. and *Carpoglossum confluens* rising above a mat of Caulerpales and a large assemblage of mixed understorey species (e.g. *Zonaria* spp. *Plocamium* spp.,). *Ulva* spp. and *Hormosira banksii* are frequently found at the low tidemark. *Phyllosphora comosa* and crustose red algae are abundant in more wave-exposed locations (Edgar 1983a, Barrett et al. 2001).

The system is subjected to multiple anthropogenic sources of nutrients including residential and industrial effluents particularly in the upper part of the Channel (North west Bay) and the Huon River. In addition, fresh water bodies (four major rivers and 36 small rivulets) drain into the Channel transport catchment and there is significant agricultural runoff (Ross & Macleod 2013). Finally, numerous salmon farms are located throughout the lower Huon and the Channel; with this area producing approximately 50% of the cultivated salmon in Australia (ca. 27,000 tonnes, ABARES, 2013).

3.2.2. Setting of experimental additions of nutrients on macroalgal communities

The ambient nutrient manipulation, experimental setup, and the monitoring and evaluation of physical factors have been described earlier for Chapter II, (pages 35 – 42).

To assess the effects of a nutrient enrichment pulse and potentially a relatively instantaneous alteration on macroalgal community development (Sensu Bender et al. 1984), algae were carefully cleared (including crustose red algae using a scraper) from the substrate within fifteen permanent quadrats in each of the 100 m^2 plots, and from nine permanent quadrats in each of the 60 m^2 plots at each location (Chapter II, Fig 2.1). Prior to clearing, the percentage macroalgal cover was estimated to provide a baseline assessment of community structure. Percentage of cover was estimated by counting the number of times each species occurred under 25 contact points within a 30 × 30 cm reticulated quadrat in five randomised quadrats in the 100 m^2 plots and in three quadrats in the 60 m^2 plots approximately every three months (Figure 3.1). Where species level identification was not possible macroalgae was assigned to a coarse taxonomic grouping (e.g., pink encrusting, red turf algae, green/ red filamentous algae). After the estimation of cover the macroalgae recruits were removed from each quadrat for subsequent identification in laboratory.

3.3.3. Statistical analysis

To observe the patterns of covariation between the identity of the most important emergent species and the potential patterns of response to treatment effects in the successional community structure, Principle Components analysis (PCO) was undertaken, with levels of similarity calculated using Euclidean similarities as the distance measures between

samples. Data was square root transformed in order to down-weight the contribution of rare taxa (Clarke et al. 2006). The contribution of each species to the observed dissimilarities was calculated using Pearson correlation coefficients, and displayed using vector diagrams on the PCO ordination. Similarity Percentage analysis (SIMPER) was used to identify the contribution of different taxa to the community composition, and distance based linear models (DISTLM) were conducted to examine the relationship of community structure and environmental drivers in each season.

3.3.4. Spatial Variability in Initial Community Analysis

The community structure at each location was analyzed prior to algal clearance and nutrient additions using a Mixed-model PERMANOVA, considering Treatment (i.e. Treatment vs. Controls, 2 levels, Fixed), Location (3 levels, random) and Plot (four levels, random). The random variation among plots was tested with the error terms of the main factors: Plot nested in treatment for Treatment, and Plot nested in Treatment × Location for Location (see Chapter 2; Table 2.1). Species abundance (% of cover) from each quadrat was aggregated into six functional groups as described for chapter II.

3.3.5. Nutrient effects on Successional Community Structure

Changes in the community structure of the early successional communities, after nutrient addition, were assessed using the Mixed-Model PERMANOVA. The model determined whether the treatment effect (i.e. T vs. C1, C2, C3) varied between locations (Table 3.1). Where the Treatment × Location interaction was significant, the tests for nutrient effects were conducted separately for each location, testing the Treatment effect against the original

error term (Plot(Treatment)). This analysis was undertaken for each season independently, because it was acknowledged *a priori* that the responses of macroalgal communities may be seasonally constrained. In addition, to investigating the effect on the composition of the whole community, separate analyses were conducted to assess the effects of nutrients on the species composition of each functional group. To establish the individual differences of functional groups between and within locations the analysis was performed with the same model.

To analyse and model relationships between the successional community structure and abiotic factors (i.e., light, wave exposure, water column nutrient, salinity and temperature) a distance based linear model constructed using Euclidean similarity (DISTLM) was undertaken. Environmental data was normalised to adjust for variation in the scale of the different variables. Akaike's selection criterion was used to evaluate the selected model. Colinearity of the abiotic data was assessed via Draftsman plots, and where high levels of colinearity were detected (e.g., $|r| \geq 0.95$) variables were arbitrarily removed from the analysis. All analyses were undertaken using PRIMER 6.0© (2006).

3.3. Results

3.3.1.Community structure before and after clearance and nutrient addition

The community structure prior to algal clearance was statistically different between locations (Table 3.1). However, no differences were observed in the number of species (3.2 A). Similarly, the analysis of functional groups showed that Fucales and Crustose/coralline algae varied significantly between locations (Table 2 B). The macroalgal community at Ninepin Point differed from the other two locations mainly as a result of the increased presence of *Phyllosphora comosa* (Fucales) and *Lithothamnion* sp. (Crustose/ coralline algae) (Figure 1 A). These species along with *Sargassum fallax* (Fucales) comprised 81 % of the community similarity at the treatment site. Whilst, *Lithothamnion* sp. and *P. comosa* accounted for 62% of the total cover (SIMPER analysis, table 3). Ordination of the successional community structure of all sites from Ninepin Point nine months after algal clearance and nutrient additions (Figure 1 B) suggests that the pattern of recruitment was similar to that observed in the adult community at pre-treatment conditions (Figure 1A).

At Tinderbox during pre-treatment conditions there was a significant presence of Caulerpales and Understorey species. The SIMPER analysis (Table 3) showed that *Caulerpa simpliscula*, *C. longifolia*, *Laurencia* sp., *Plocamium* sp. and *Zonaria* sp. were common species across plots. Caulerpales represented more than the 70% of the community and played a key role in differentiating this community from other locations (Table 3 A). *Ecklonia radiata* and *Sargassum fallax* were also abundant at Tinderbox but presented a patchy distribution ($\delta_i/SD(\delta) < 1$) across all plots.

The structure of Green Island's assemblage would appear to be intermediate to these other communities (Figure 1 A, B). At this location *Caulerpa trifaria*, *Caulerpa simpliscula*, turf algae, and *Lithothamnion* sp. defined the algal assemblages (66% of community similarity). Turf-forming algae were the only opportunistic group observed at Green Island, and constituted approximately 8% of the similarities at the treatment site and 11% in the control sites (Table 3 B). The community structure at the treatment site in Ninepin Pt. was notably defined by *Lithothamnion* sp. (74% of the overall trend) and *Sargassum fallax* (7% of the overall trend) and by *Lithothamnion* sp., *P. comosa* and *Sargassum verruculossum* in control sites making up 46, 16 and 12% of the overall contributions respectively (Table 3 C).

3.3.2. Nutrient water column

The effect of the addition of nutrients to the water column at each of treatment plots has already been described and discussed in chapter II (See Figures 5, 6 and Table 7, Chapter II)

3.3.3. Effect of nutrient additions on macroalgal community development

The nutrient additions had no apparent effect on either the early community structure (Table 5) or the number of species at the treatment sites (Table 5). Whilst there was a high degree of variability at the treatment sites, this was still within the range of variation observed at the controls during the course of the experiment (Figure 2 A, B, C). However, the community structure clearly differed between locations during the three sampling seasons, and this difference would seem to underpin the responses of the early community composition. The PCO analysis shows a clear gradient in the pre-clearing communities across locations, with greater overlap between the successional communities at Tinderbox and Green Island compared with Ninepin Pt. The

community at Ninepin Pt. was notably different from the other locations during winter (Figure 2 A; 45.3% of explained variation). However, during spring these differences were mostly driven by an important recruitment of unidentified branched red algae at the treatment site (Table 7), which distinguished the consistent presence of *Lithothamnion* sp. and *P. comosa* in the controls (Figure 2 B). In summer there was a shift in the community composition, where the treatment site at Ninepin Pt. was dominated only by *Lithothamnion* sp. while control sites exhibited a more diverse assemblage composed by *P. comosa* and *Haliptilum roseum*. However, the substrate seemed to be monopolized by crustose algae (Figure 2 C; Table 7). The average abundance of functional groups that characterised the community structures throughout the sampling seasons showed high small-scale variability between plots, but clearly evidenced the separation of successional patterns between locations (Figure 3). This was particularly apparent in the distribution of Caulerpales (Figure 3 A) and Laminariales (figure 3 C) at Tinderbox and Green Island, and Crustose/ coralline algae at Ninepin Pt. (Figure 3 F).

3.3.4. Patterns of early successional macro-algae community between locations

Tinderbox

The SIMPER analysis indicated that in winter, Caulerpales (*C. simpliscula, C. longifolia*), *Cladophora federayi* and *Rhodymenia* sp. contributed most to the early recruitment process at the treatment site, representing 60, 17 and 16% of the overall cumulative contribution respectively. At the control sites, understorey species, opportunistic species (*C. federayi* and turf algae) and Caulerpales were amongst the first colonisers, with 55, 30 and 9% of the overall contributions (Table 6 A). In spring, the Laminaria *Ecklonia radiata*

and the fucoid *Sargassum fallax*, emerged as the most important components of the successional community in both treatment and control sites. No opportunistic species were detected (Table 6 B). Progressing into summer, although *E. radiata* remained dominant (30 and 25% of total cover in both treatment and control plots), *C. simpliscula* became more common contributing 26% to the total contributions Opportunistic and fast-growing species were more abundant in this season increasing its abundance to a 37% in the treatment site and to a 15% at control sites, but still had only sparse spatial distribution with high small-scale variability evidenced by the ratio between average contribution/SD in the samples below 1 (Table 6).

Green Island

Turf algae, *Lithothamnion* spp., *Ulva* spp., Caulerpa simpliscula and *C. trifaria* were the most common and consistent taxa, and their occurrence was an important discriminating feature between locations (Table 6 D). Turf algae and *U. australis* accounted for 40% of the general contributions in winter. Whilst, *Lithothamnion* sp. comprised only 26%. This trend diverged from the other locations. In spring, *Ulva*, emerged as the most abundant species in the treatment site accounting for 33% of the overall cumulative contribution. However, *Lithothamnion* spp. and *Sargassum vestitum* accounted for the 40 and 26 % of the total similarities. In control areas, *C. simpliscula* contributed 20% to the overall group similarity, but opportunistic species (Filamentous brown algae, *Ulva* spp. and ephyphitic species) comprised the 40% of the overall cumulative contribution. During summer, there was a shift in the treatment site where *Lithothamnion* spp. contributed with the 60% and Caulerpales (*C. trifaria* and *C. simpliscula*) with the 37%. Opportunistic species, represented by turf-forming species constituted 13% of the total contributions. However, in control sites turf-forming algae were the most

abundant species (53% of the general cumulative contribution), while *Lithothamnion* sp. contributed with a 25% and Caulerpas provided the 14% of the total cover (Table 6 F).

Ninepin Pt.

The successional community at Ninepin Point appeared to have several, species (*Lithothamnion* sp.1 and 2 and *Phyllosphora comosa*) which were relatively stable and consistent over time, and this helped differentiate it from the other two locations (Figure 3 D and E). Crustose algae (*Lithothamnion* sp.1 and *Lithothamnion* sp.2) were the most conspicuous functional group throughout the study period; comprising more than 90% of the community similarity at both treatment and control sites in winter Table 6 G). In spring *Lithothamnion* spp., comprised 67% of the overall community similarity at the control sites, where juvenile specimens of the canopy-forming fucoid *Phyllosphora comosa* contributed only 20% to the overall community similarity (Table 6 I). Unidentified branched red algae were conspicuous in all samples from the treatment site, reaching around 100% of the individual specie's contribution (Table 6 H). During summer Crustose algae and *P. comosa* comprised about 90% of the overall community similarity at the control sites, but *Lithothamnion* was the only species present at the treatment plot.

3.3.5. Influence of environmental variables in successional community

There was no clear relationship between environmental factors and the treatment effect in the early successional stages of macro-algae at any location. The patterns of colonisation and succession mirrored the adult community at all sites, and reflected the environmental conditions at those sites regardless of successional stage. Having said that, wave exposure

(Openness) and temperature were consistently associated with the community structure of Ninepin Pt. suggesting a link between these environmental conditions and algal succession (Figure 4 B and C). Accordingly, the spatial occurrence of *Lithothamnion* sp. *and P. comosa* at Ninepin Pt. would appear to be associated with wave exposure across seasons. However, wave exposure itself explained little of the total variability (1% in winter, 4% in spring and 5% in summer). A similar relationship was observed with temperature (21, 13, and 10 % of total variability in winter, spring and summer respectively). DIN also seems to be associated with the separation of Ninepin Pt. at the low Channel sites. However, it too explained little of the total variability during the three seasons (1.6, 4 and 4 % respectively). Light (13% of total variability) and at lesser extent salinity (2% of total variability) appeared to be the major drivers of the mid channel assemblages during spring, but not at other times (Figure 4 B). However, this may be a function of the relationship with the annuals *Ulva* and Caulerpales, which reached peak abundances at this location during this season (Figure 3 A and F).

Table 3. 1. Mixed-model PERMANOVA analysis comparing the macroalgal community structure throughout locations during pre-treatment conditions (Autumn).

Source	Df	SS	MS	Pseudo-F	p-value	Permutations
Treatment	1	7506.8	7506.8	1.1	0.4	9934
Plot(Treatment)	2	17217	8608.7	0.78	0.6	9925
Location	2	92351	46176	3.28	0.01	9946
Treatment × Location	2	16931	8465.7	0.64	0.7	9943
Location × Plot(Treatment)	4	44057	11014	4.06	p<0.01	9880
Residual	130	3.5E5	2710.7			
Total	141	5,3E5				

Table 3. 2. Summary of Mixed-model PERMANOVA comparing macroalgal functional groups and Two-way ANOVA of number of species (Richness) throughout locations during pre-treatment conditions (Autumn). (**) Indicates p < 0.01, (*) indicates p < 0.05.

A. Richness	Treatment		Location		T × L		Plot(T)		Location×Plot(T)	
	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df
	--	--	9.4	2	0.2	2	0.6	1	3.2*	4
B. Functional group	Treatment		Location		T × L		Plot(T)		Location×Plot(T)	
	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df	Pseudo-F	df
Caulerpales	1.2	1	2	2	0.9	2	0.65	2	1.8**	4
Understorey	0.3	1	2	2	0.7	2	0.95	2	1.7**	4
Opportunistic spp.	1	1	1	2	0.6	2	1	2	2.1**	4
Laminariales	0.8	1	1.9	2	0.9	2	1.3	2	1	4
Fucales	1	1	2*	2	0.5	2	0.7	2	3**	4
Crustose/Coralline	1.3	1	3.2*	2	0.6	2	0.5	2	2**	4

Table 3. 3. Summary of SIMPER analysis per location showing the relative contribution to group similarities (%), proportion of total abundance within community group (%) of species that contributed with the 90% to overall groups similarity during pre-disturbance conditions (autumn) for every location and condition.

A. TINDERBOX				
Treatment; Average similarity: 28.60				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Caulerpa simpliscula	3.73	0.93	39.92	39.92
Caulerpa longifolia	3.31	0.48	24.17	64.09
Ecklonia radiata	2.29	0.44	12.62	76.71
Sargassum Fallax	1.58	0.32	7.85	84.56
Caulerpa trifaria	1.48	0.37	7.61	92.17
Controls; Average similarity: 20.76				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Laurencia sp.	2.05	0.47	19.43	19.43
Plocamium angustum	1.35	0.43	12.57	32
Zonaria sp.	1.31	0.47	12.42	44.42
Lithothamnion sp. 2	1.51	0.38	11.99	56.4
Sargassum Fallax	1.54	0.39	11.57	67.97
Caulerpa longifolia	1.59	0.2	8.44	76.41
Lenormandia marginata	1.28	0.26	7.32	83.73
Ecklonia radiata	1.21	0.26	5.51	89.24
Caulerpa trifaria	0.9	0.19	3.14	92.37
B. GREEN ISLAND				
Treatment; Average similarity: 14.54				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Caulerpa trifaria	2.03	0.4	21.26	21.26
Caulerpa simpliscula	1.96	0.39	19.77	41.04
Lithothamnion sp.	1.26	0.37	12.76	53.79
Lithothamnion sp. 2	1.26	0.3	11.91	65.7
Turf algae	1.16	0.3	7.34	73.04
Sargassum vestitum	1.15	0.23	6.9	79.94
Caulerpa brownii	1.38	0.17	6.25	86.19
Ecklonia radiata	0.97	0.24	5.7	91.89
Controls; Average similarity: 19.77				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Caulerpa trifaria	3.46	0.49	41.56	41.56
Turf algae	1.99	0.33	11.64	53.2
Sargassum vestitum	1.46	0.33	10.45	63.65
Lithothamnion sp.	1.2	0.33	8.05	71.7
Caulerpa simpliscula	1.38	0.3	7.67	79.37
Ecklonia radiata	0.94	0.29	5.65	85.02
Red recruits	0.98	0.2	3.48	88.5
Caulerpa brownii	0.82	0.13	1.92	90.42
C. NINEPIN Pt.				
Treatment; Average similarity: 36.85				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Lithothamnion sp.	6.03	1.2	73.96	73.96
Sargassum Fallax	1.41	0.48	7.37	81.33
Zonaria sp.	1.11	0.32	4.43	85.76
Cystophora retroflexa	1.19	0.31	4.07	89.84
Sargassum verruculossumm	1.08	0.23	2.66	92.5
Controls; Average similarity: 31.77				
Species	Av.Abund.	δ/SD(δ)	C(%)	Cum. %
Lithothamnion sp.	4.67	0.96	46.44	46.44
Phyllosphora comosa	2.49	0.56	16.03	62.47
Sargassum verruculossumm	2.25	0.47	11.96	74.43
Zonaria sp.	1.64	0.62	10.89	85.32
Lithothamnion sp. 2	1.32	0.36	4.87	90.19

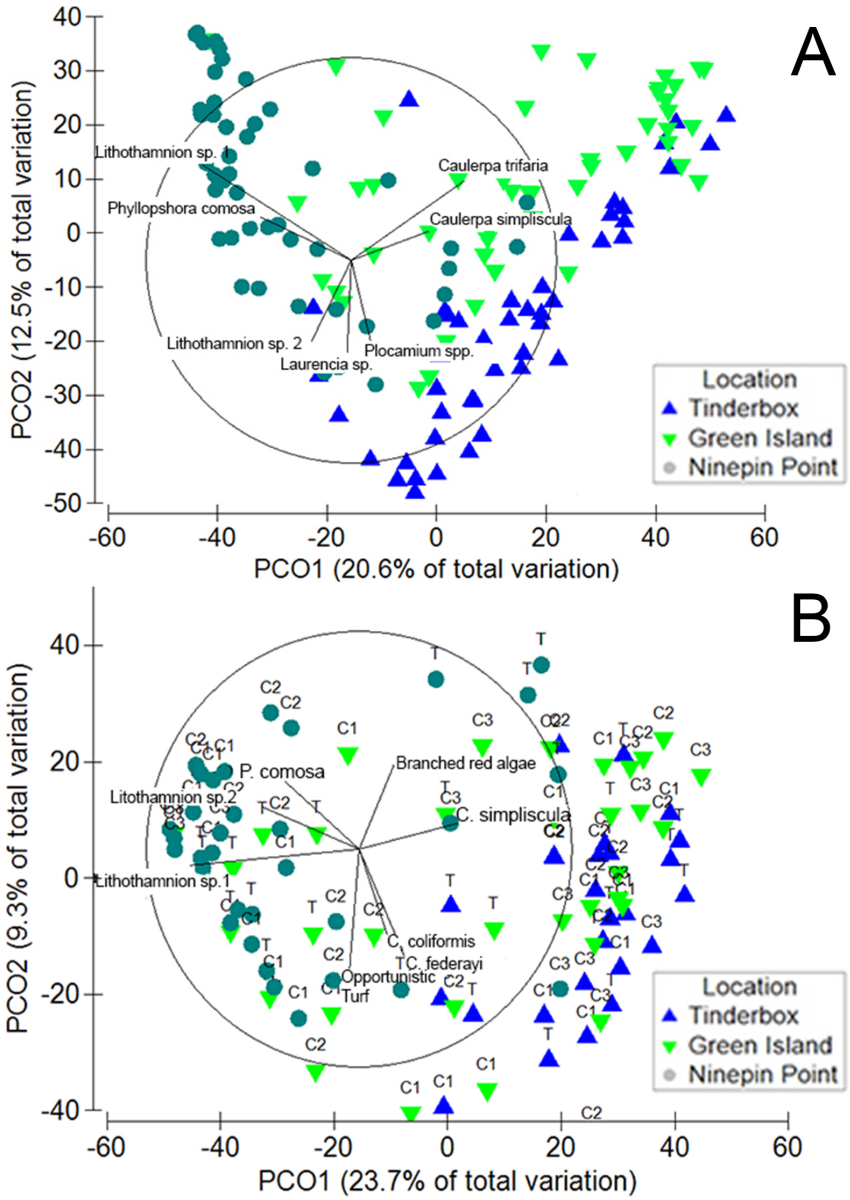

Figure 3. 1. Principal Coordinates Ordination (using Bray-Curtis dissimilarity) on (A) adult macroalgal communities (33% of the variation explained) prior to algal removal and nutrient addition and (B) accumulated successional community structure after 9 months post algal removal and nutrient addition for individual replicates samples at each of the three study locations (n = 140). The vectors show Spearman correlation (r > 0.5) for the species best correlated with the first two axis (Circle indicates a radius of r = 1).

Table 3. 4. Summary of mixed model PERMANOVA on the effect of nutrient addition on the early successional macroalgae community for winter, spring and summer. (*) Indicates p-values <, = 0.05; (**) < 0.01.

Source	Winter		Spring		Summer	
	F	p-value	F	p-value	F	p-value
Treatment	1.3	0.2	0.7	0.7	0.8	0.6
Location	3.6	p< 0.01	3.1	p< 0.01	2.9	0.02
Treatment × Location	0.9	0.5	2	0.7	1	0.4
Plot(Treatment)	0.7	0.8	1	0.5	1	0.5
Location*Plot(Treatment)	1.7	p< 0.01	1.6	p< 0.01	1.2	0.3

Table 3. 5. Summary of mixed model PERMANOVA on the effect of nutrient addition on number of species during winter, spring and summer. (*) Indicates p-values <, = 0.05; (**) < 0.01.

Source	Winter		Spring		Summer	
	F	p-value	F	p-value	F	p-value
Treatment	---	---	0.6	0.5	0.6	0.5
Location	3	0.5	1.8	0.3	1	0.5
Treatment × Location	0.4	0.7	3.1	0.1	---	---
Plot(Treatment)	0.1	0.9	0.5	0.6	5.4	0.1
Location*Plot(Treatment)	5.1	p< 0.01	1.3	0.3	0.1	0.8

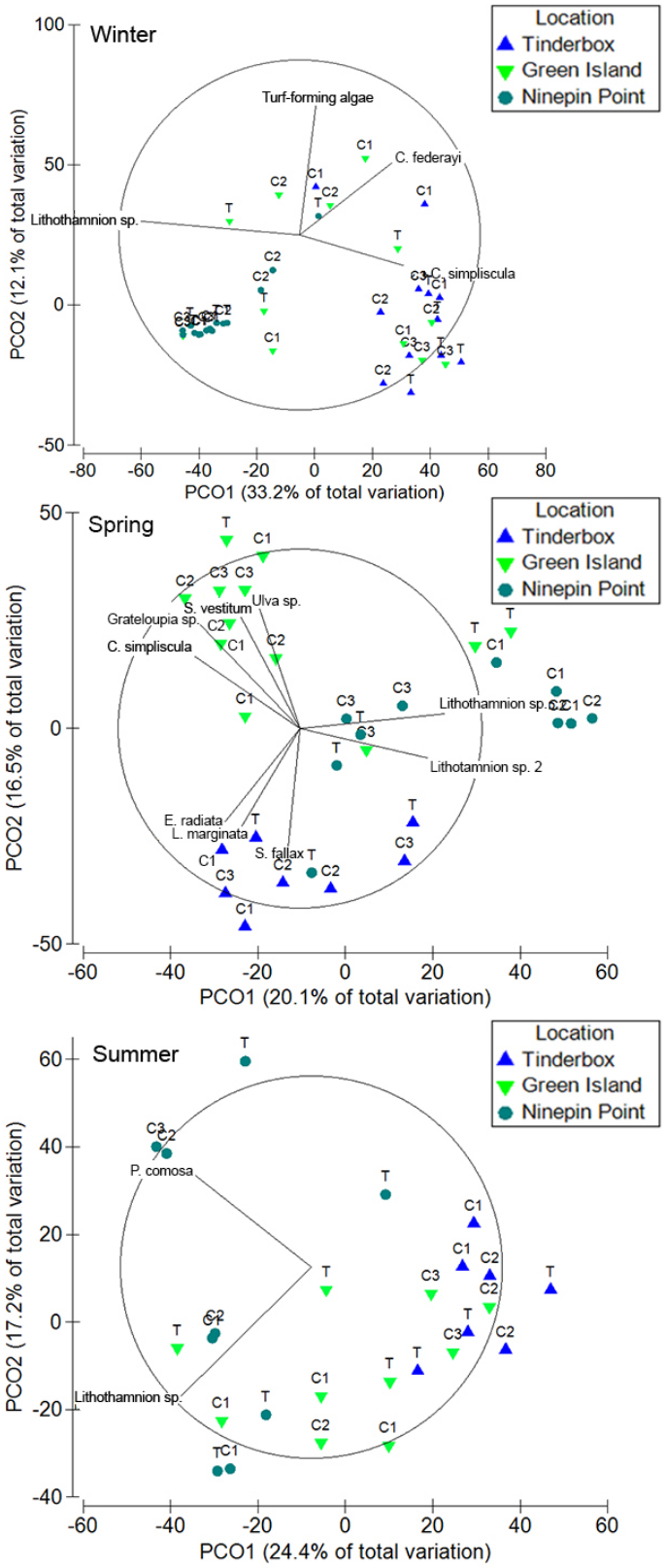

Figure 3. 2. Principal Coordinates Ordination using Bray-Curtis dissimilarity on community development three macroalgal reefs during winter (upper panel), spring (middle panel) and summer (Lower panel). T = quadrats from treatment plots. C1, C2 and C3 = quadrats from control plots. The vectors show Spearman correlation (r > 0.6) for the species best correlated with the first two axis (Circle indicates a radius of r = 1).

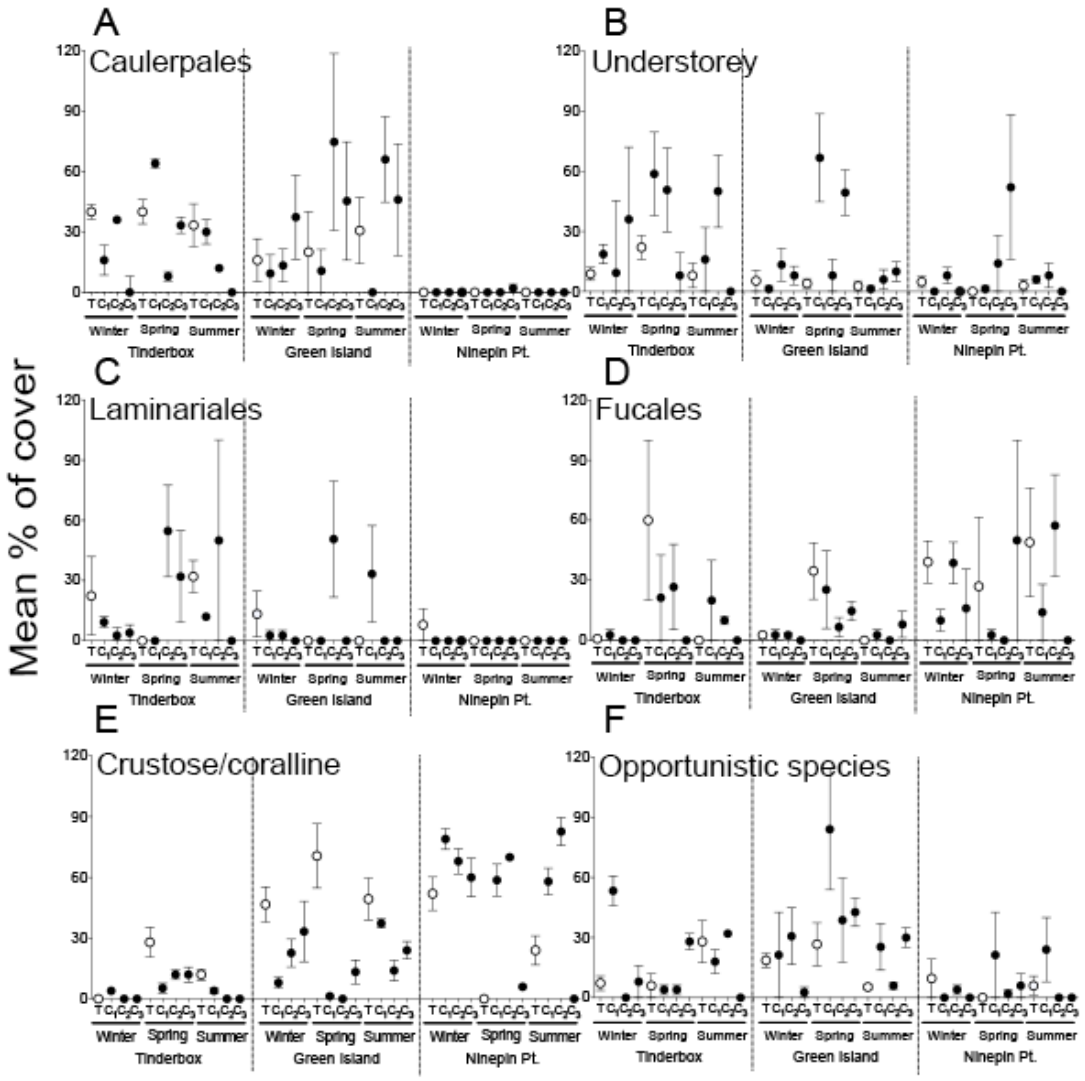

Figure 3. 3. Mean abundance (% of cover ±SE) of functional group at each site season and treatment (T = treatment/clear circles; C1, C2, C3 = controls/solid circles

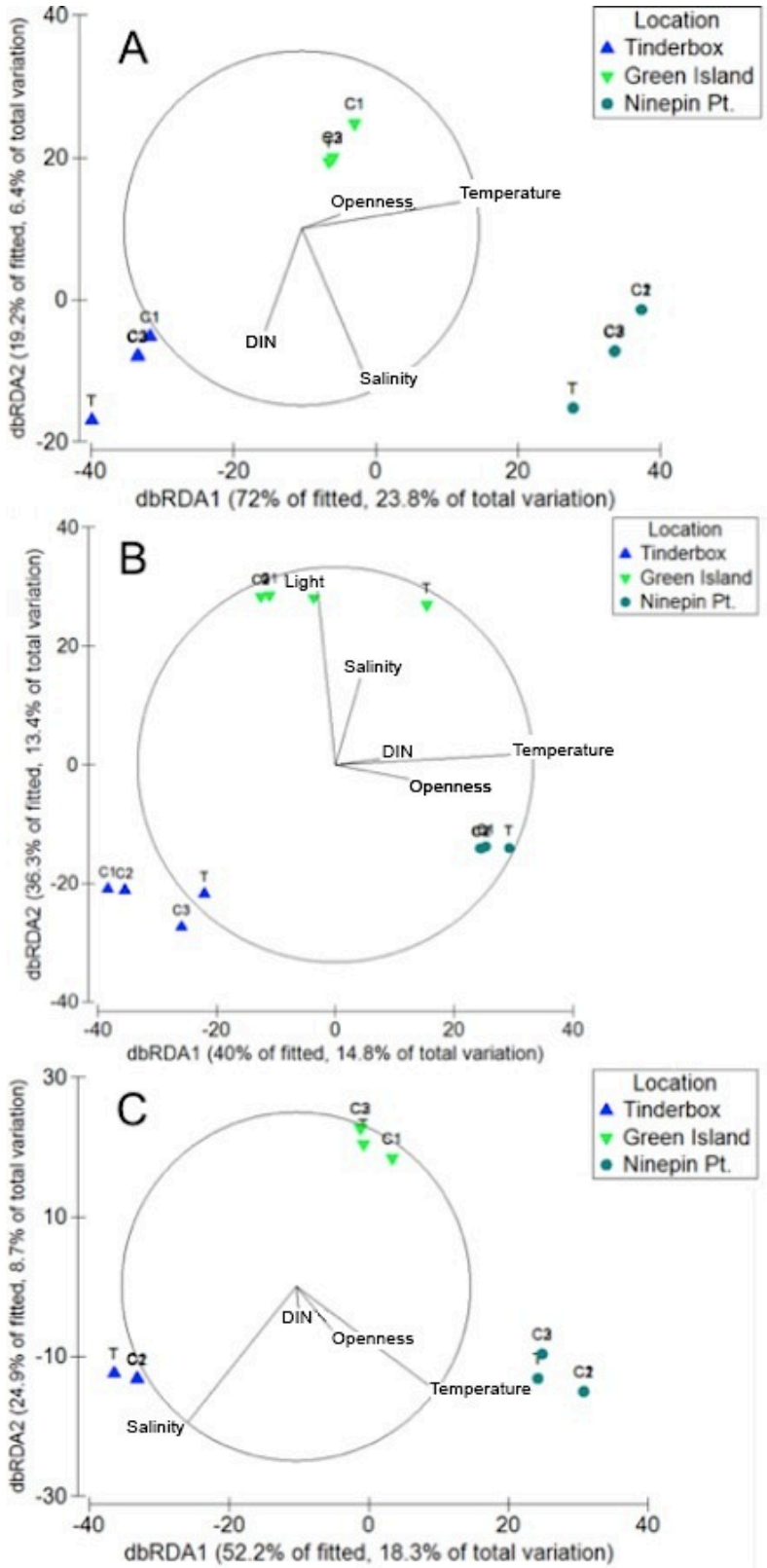

Figure 3. 4. Distance-based RDA ordination of abiotic factors during (A) after 3 months (winter; n = 39), (B) 6 months (spring; n = 31) and (C) 9 months (summer n = 26) of disturbance exposed to experimental nutrient additions. T = treatment sites, C1, C2, C3 = Control sites.

Table 3. 6. Summary of SIMPER analysis showing those macro-algal species that contributed most to the successional patterns in each season, location. (F_T%) Frequency in total sample, (C%) Individual contribution of species (cut off 90%) to the overall group similarity. (δ_i/SD(δ)) ratio of average contribution and SD of those contribution across pairs of samples.

A. Tinderbox winter					D. Green Island winter					G. Ninepin Pt. Winter				
Treatment; Average similarity: 38.06					Group T; Average similarity: 35.98					Treatment; Average similarity: 52.76				
Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%
C. simpliscula	3.55	3.32	53.54	53.54	Ulva sp.	3.15	3.39	28.08	28.08	Lithothamnion sp.1	6.63	1.71	90.87	90.87
C. ferderayi	2.03	0.61	16.97	70.51	Lithothamnion sp.	4.77	0.58	26.38	54.46					
Rhodymenia sp.	1.82	0.61	16.97	87.49	Turf algae	2.79	0.58	12.48	66.94					
C. longifolia	1.96	0.32	5.56	93.05	C. simpliscula	2.1	0.58	10.25	77.2					
					Lithothamnion sp. 2	2.3	0.58	8.34	85.54					
					C. brownii	1.33	0.58	7.25	92.79					
Controls; Average similarity: 14.65					Controls; Average similarity: 17.39					Controls; Average similarity: 48.69				
Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%
Zonaria spp.	1.43	0.6	20.62	20.62	C. trifaria	2.23	0.53	34.73	34.73	Lithothamnion sp.1	6.1	2	68.82	68.82
C. ferderayi	2.34	0.39	18.87	39.48	Lithothamnion sp. 2	1.53	0.33	16.88	51.61	Lithothamnion sp. 2	24	0.83	27.37	96.19
Plocamium spp.	0.98	0.38	17.29	56.77	Turf algae	3.21	0.3	14.18	65.79					
L. marginata	0.86	0.38	11.53	68.3	Lithothamnion sp.	2.11	0.24	9.72	75.51					
Turf algae	2.87	0.22	10.89	79.19	C. federayi	0.99	0.3	6.33	81.84					
C. longifolia	1.83	0.22	8.83	88.02	Champia viridis	0.98	0.29	4.84	86.68					
Rhodymenia sp.	0.81	0.22	4.66	92.68	C. geminata	1.16	0.17	4.25	90.93					
B. Tinderbox Spring					E. Green Island Spring					H. Ninepin Pt. Spring				
Treatment; Average similarity: 42.91					Treatment; Average similarity: 40.75					Treatment; Average similarity: 46.00				
Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%
S. Fallax	7.24	0.6	32.9	32.9	Ulva sp.	4.67	11.84	33.34	33.34	Branched red algae	2	1.69	100	100
Red algae recruits	4.38	0.9	25.48	58.38	S. vestitum	3.61	8.5	26.51	59.85					
Lithothamnion sp.2	4.58	0.3	20.81	79.19	Lithothamnion sp. 2	5.5	0.58	23.81	83.67					
C. simpliscula	4.73	0.7	20.81	100	Lithothamnion sp.	3.77	0.58	16.33	100					
Controls; Average similarity: 40.12					Controls; Average similarity: 22.42					Controls; Average similarity: 28.60				
Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%
E. radiata	6.32	4.23	41.91	41.91	C. simpliscula	3.52	0.59	20.68	20.68	Lithothamnion sp.	4.36	0.78	40.92	40.92
S. Fallax	3.88	0.78	15.73	57.64	Filamentous brown	2.47	0.57	17.76	38.43	Lithothamnion sp.2	3.2	0.88	26.62	67.54
L. marginata	3.24	0.78	12.73	70.37	Ulva sp.	3.06	0.58	15.2	53.63	P. comosa	4.23	0.39	21.17	88.7
C. geminata	3.11	0.7	10.13	80.51	Grateloupia sp.	2.28	0.42	7.78	61.41	Zonaria spp.	0.86	0.39	4.68	93.39
Lithothamnion sp.2	1.88	0.48	4.92	85.43	Red recruits	2.32	0.39	6.82	68.23					
Red algae recruits	2.33	0.46	4.52	89.96	S. vestitum	1.8	0.42	6.57	74.8					
C. trifaria	1.73	0.46	4.39	94.35	Epiphytic load	1.77	0.27	6.34	81.15					
					C. trifaria	2.16	0.29	6.25	87.4					
					Cystophora retroflexa	1.37	0.3	3.35	90.75					
C. Tinderbox Summer					F. Green Island summer					I. Ninepin Pt. summer				
Treatment; Average similarity: 53.96					Treatment; Average similarity: 38.95					Treatment; Average similarity: 7.27				
Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%
E. radiata	6.12	3.48	29.58	29.58	Lithothamnion sp.	3.1	2.84	30.07	30.07	Lithothamnion sp.	3.31	0.41	100	100
C. simpliscula	5.04	7.15	26.83	56.41	Lithothamnion sp. 2	5.06	0.58	29.34	59.42					
Ulva sp.	2.76	6.12	14.54	70.95	C. simpliscula	3.6	0.58	16.86	76.28					
C. coliformis	2.28	13.72	12.87	83.82	Turf algae	2.79	0.58	13.06	89.34					
C. federayi	2.97	0.58	8.09	91.91	C. geminata	2.28	0.58	10.66	100					
Controls; Average similarity: 25.38					Controls; Average similarity: 32.16					Controls; Average similarity: 39.37				
Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%	Species	Av.Abund.	δi/SD(δ)	C(%)	F_T%
E. radiata	4.23	0.89	25.25	25.25	Turf algae	5.56	4.06	53.21	53.21	Lithothamnion sp.	4.65	1.61	36.22	36.22
Zonaria spp.	2.85	0.91	21.02	46.27	Lithothamnion sp.	2.93	0.61	18.6	71.8	Lithothamnion sp.2	5.37	0.8	27.39	63.61
S. Fallax	3.15	0.9	18.5	64.76	C. trifaria	2.3	0.35	7.92	79.72	P. comosa	5.01	0.85	24.86	88.47
C. billardieri	1.73	0.41	9.44	74.21	Lithothamnion sp. 2	1.63	0.39	6.68	86.4	H. roseum	1.2	0.62	7.19	95.66
Plocamium sp.	1.73	0.41	6.64	80.85	C. simpliscula	2	0.34	5.39	91.79					
C. federayi	1.57	0.41	5.42	86.28										
L. marginata	1.41	0.41	5.42	91.7										

3.4. Discussion

This study showed that experimental enrichment did not have a measurable effect on the community composition at any of the study sites or at any particular time, and did not result in a marked increase in colonization of opportunistic fast-growing macroalgae after clearing. There are two logical interpretations for these outcomes. 1) The intensity of the nutrient pulse of enrichment was not strong enough to cause a significant change in the community trajectory or an early replacement that may suggest, in turn, a change in function; 2) the level of disturbance (clearings) was not pronounced enough to allow opportunist's colonisation and consequently the response was masked by other physical or biological key factors that drive the variability at the spatial scale of this study.

The nutrient additions were shown to significantly elevate nutrient concentrations adjacent to the source (i.e. within 10cm) but this increase was quite moderate when measured at 1m (see discussion in Chapter II for further interpretation). Consequently, it may be surmised that the inherent variability and resilience of these communities may have masked or mitigated the sort of early signals associated with the added nutrient that have been observed in previous studies elsewhere (Bokn et al. 2003, Karez et al. 2004). The high variability observed in the developing assemblages may have been promoted by differences in water flow between locations, as this has been shown to have a great influence on propagule transport and colonisation success in Southern Australia (Wernberg & Connell 2008, Smale et al. 2011); this can also be enhanced by the topography of reefs.

The results also suggest that colonisation patterns responded to location-specific environmental differences with green foliose, filamentous and turf-

forming species being a common component at sites in the upper and mid Channel (i.e., Tinderbox and Green Island), but were less frequently present at the lower channel location (Ninepin Pt.). We suggest that a coupled effect of reduced levels of wave exposure and differences in light levels at Green Island may have facilitated the occurrence and permanence of turf-algae at this location. In contrast, the "canopy-encrusting algae" recruiting assemblage at Ninepin Pt. (i.e. *Lithothamnion* spp. and *Phyllosphora comosa*) may be responding to the higher level of wave action at this site. Such communities have been associated with sites subjected to increased wave-exposure (Steneck 1986, Connell 2003, Wernberg & Connell 2008, Smale et al. 2011, Marzinelli et al. 2015). Crustose algae have been shown to limit substrate availability, decrease the occurrence of crevices (< rugosity) and reduce protection against wave action or grazing once spores arrive (Dudley & Dantonio 1991). This in addition to the scouring effects of canopy-forming algae from neighbouring areas can create adverse conditions for the recruitment of species such as opportunistic turfs (Kiirikki 1996, Connell 2003). The sites at Tinderbox had a more sheltered habitat (Barrett et al. 2001), and as such were more widely covered with Caulerpales (Edgar 1983, 2008), which readily colonised available substrate, which in turn may have prevented the settlement of opportunistic and turf-forming species at this location.

Whilst wave exposure will increase the levels of mechanical stress, scouring, transport and settlement of spores and propagules (Kraufvelin et al. 2002, Denny 2006, Wernberg & Connell 2008, Smale et al. 2011), local differences in light may also provide a very plausible explanation for the community development observed. The tannin stained water of Huon River has been shown to significantly influence the local macroalgal ecology at the lower

Channel, and particularly at Ninepin Point (Barrett et al. 2009). Accordingly, Green Island sites in the mid Channel area, which are less influenced by the Huon river conditions, may have favoured the persistence of opportunistic macro-algae.

Variable salinity regimes exist within the study region, due to the presence of freshwater streams in the upper and lower Channel (Ross & Macleod 2013) This may have an adverse effect on the productivity and nutrient uptake of opportunist germlings. Detrimental effects on the recruitment of fast-growing species such as *Enteromorpha intestinalis* and *Ulva expansa* exposed to decreased salinity regimes have been demonstrated experimentally (Fong et al. 1996, Kamer & Fong 2000). Experiments have also shown that there can be synergistic effects associated with increases in nutrients, light and salinity, whereby spore growth and productivity in *Ulva* and *Enteromorpha* were enhanced (Lotze et al. 2000). However, whilst relatively easy to show in the laboratory, such relationship are much harder to demonstrate in the context of highly variable estuarine systems (Wolanski & Elliott 2016). Accordingly, understanding the effects of extreme variations in salinity on macroalgal assemblages at local level may need further attention.

Although light levels were correlated with the succession patterns observed from the mid-Channel sites, inferring a link between light and the successional response should be done with caution. The light environment in estuarine systems is also highly affected by hydrodynamic forces (i.e. tides, currents, mixing of fresh water, seawater and winds), which can result in large-scale changes in resuspension of sediment, organic matter and phytoplankton abundance (Duarte 1995, Dixon et al. 2014). Consequently, a more comprehensive survey of the coupled effects of light availability and

physical drivers may be needed to determine the validity of any location-specific effects associated with the light environment.

Temperature and dissolved inorganic nutrients have also been shown to be highly influenced by water motion and freshwater inputs, with a number of studies showing strong spatial differences in the patterns of succession associated with wave exposure (Lubchenco & Menge 1978, Kawamata 1998, Kamer & Fong 2000). Whilst this relationship is often highly location-specific, it is clear that wave exposure and freshwater inflows would be important primary factors to consider when monitoring nutrient effects on algal communities, and that they could also provide a valuable spatial context to identify/ define community sensitivity.

In this study the developing community showed a tendency to return to initial conditions. In circumstances where the level of disturbance is moderate (as appears to be the case in the present study) the initial community structure following clearings may reflect the composition of the surrounding adult community. Experimental evidence has shown that the surrounding environment can act as a source of propagules, which would allow recolonisation of the particular species from the adjacent community (Connell & Slatyer 1977). In other words, the species mix present nearby would in large part drive the successional community. In this study as opportunists were not a major component of the background communities, and then there was not a ready supply of propagules for these species. However, the "neighbour colonisation" concept may be an over simplification of more complex interactions that facilitate selective recruitment, such as disturbance events modulated by water motion, herbivory and competition with other macro-algae (Edgar 1983a, Steneck 1986, Connell 2003).

The persistence of opportunistic turf-forming algae at Green Island may reflect a resident source of propagules in the mature community and

appropriate conditions that facilitate their growth. Opportunistic turf-forming algae are common colonising species (Connell et al. 2014), and are widely recognised as indicative of organically enriched environments (Gorgula & Connell 2004, Russell et al. 2005, Connell et al. 2014).

On the other and, it is important to note that there was a significant proportion of variation at all sites that could not be explained by the physical drivers alone, and there may be other factors that affect the abundance of opportunistic algae between locations. For instance, grazing and sedimentation rates (not assessed here) can have major effects on recruitment and survival of macro-algae (Underwood & Fairweather 1989, Santelices 1990). Previous studies in the NW Atlantic (Lotze et al. 2001), the Baltic sea (Eriksson et al. 2007) and South Australia (Russell & Connell 2005, 2007) have all shown that grazing pressure may be critical determinant of the community structure where there is moderate nutrient enrichment (top-down effects). There are studies that have suggested that grazing and disturbance interactions can account for poor reponses to nutrient fertilization (Bokn et al. 2003, Karez et al. 2004). However, there is also evidence to suggest that grazing effects may have little impact in algal communities in South Australia (Lavery & Vanderklift 2002). The role of the coupled effect of herbivory, physical disturbances (clearings) and nutrient enrichment in algal succession at local spatial scales deserves further attention (Reed & Foster 1984, Bertness et al. 1999, Wikstrom & Kautsky 2007). Further research is also needed on the combined effect of these variables and prevailing environmental drivers. This may provide additional insights for coastal management, particularly if such research can put the community response to nutrient fertilization in the context of spatial variability.

This study highlights the inherent resilience of temperate reef habitats under real world conditions, but also emphasizes the broad-scale influence of environmental variability and location-specific differences in the response of early colonisation. This emphasizes that the sensitivity of shallow rocky reefs to nutrient impacts may present differences in the pattern of succession at spatial scales of tens of kilometres, where the sensitivity to prevailing biophysical conditions may play a crucial role. This is particularly relevant for the monitoring and the estimation of indicators of nutrient enrichment and impacts, as location-specific biophysical differences may be sufficient to counterbalance or diminish the community response to clearing/ nutrient addition.

The lack of response in the current study may also be that the scale of sampling was not sufficient to detect the change, i.e. any impact was masked by a combination of post-temporal and random variation. A pulse perturbation, such as employed in this study, may cause an immediate alteration in species abundance however, given such characteristics the system can return to its previous equilibrium state very quickly (Bender et al. 1984). This suggests that further understanding is needed both the immediate reaction (to pulse perturbations) and the longer-term response (to press perturbations) to nutrient additions, and how this might affect productivity, particularly in field experiments. Although whole community field based experiments may carry substantial uncertainty, evaluation undertaken at this scale is critical to understanding broad-scale ecosystem function, and to developing “realistic” management strategies and a predictive capability.

Whilst this study has identified some important considerations for monitoring and management we have to acknowledge that there were some constraints in the sampling design that will limit i) the extent that we can generalise the findings and ii) in some instances the certainty we can place on conclusions

(level of type I error). Unfortunately, the high levels of spatial variability (both within and between locations) seem to weaken further inferences. This may indicate insufficient spatial replication to confidently define the nutrient effect or effectively determine any differences in broader physical patterns. This stresses the importance of replication in separating a stress signal from the background spatial variability (Chapman et al. 1995).

Overall, the findings do allow some prediction on areas of vulnerability. Reefs exposed to increased wave action may represent greater initial resistance to nutrient fertilization due to greater disturbance, mechanical effects and transport of propagules. Similar effects may be driven in these sites through, shading, scouring and substrate constraints driven by Canopy-forming and crustose species. Conversely, it seems that more sheltered reefs showed different patterns of succession that may have facilitated turf-forming algae in particular at the mid Channel. In turn, this suggests that these reefs may be more susceptible to nutrient fertilization as a function of habitat conditions (e.g., low wave action associated with differences in light attenuation and an underlying propagule sources).

In conclusion, the early successional community composition responded to location-specific species relationships and the broad-scale environmental conditions that structure these. This suggests that the underwater vegetation in this system still might have a certain level of resilience, with a high probability to be replenished from neighbouring adult community (progeny) after small-scale disturbance. Accordingly, underlying abiotic and ecological interactions may compensate or override the effects of nutrient additions on macro-algae succession. In this sense, the use of successional stages to estimate the system response in ecosystems in early phases of eutrophication may require further research. Recruitment is a critical stage in

community development and can be influenced by wide range of physical and biological factors, which in turn, act at different temporal and geographical scales (Underwood & Fairweather 1989, Coelho et al. 2000, Lotze et al. 2000). However, it is suggested that a combined understanding of both the physical attributes of the system and the successional community composition is necessary to provide a broad-scale environmental context and that this would be essential for any prediction of the system's ability to respond to nutrient enrichment. Ultimately, these results suggest that to clearly, quickly and accurately determine the eutrophication status and environmental risk, there is a need for alternative indicators that highlight sensitivity to impacts caused by nutrient enrichment. These indicators should provide a long-term indication (cumulative) of changes in patterns of nutrient availability and that might be promoted by the same environmental attributes that increase the spatial variability of ephemeral species, for example, perennial species of macro-algae.

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Chapter 4. Physiological evaluation of the effect of nutrient loads on macroalgae as early indicator of nutrient enrichment

4.1 Introduction

In estuarine systems, nutrient inputs from anthropogenic activities can lead to the appearance of 'bloom-forming', fast-growing 'opportunistic' species of macroalgae (Russell et al. 2005, Gorman et al. 2009, Oh et al. 2015). In high abundances these species can alter habitat structure and biodiversity of reef ecosystems with significant costs for tourism, fisheries and biodiversity (Krause-Jensen et al. 2008, Gorman & Connell 2009). Because the occurrence and dominance of bloom-forming species of macroalgae is linked with habitat deterioration these species have traditionally been used as indicators to provide information about the status of coastal environments (Salas 2006, Dauvin et al. 2010)

In their review, Salas et al. (2006), suggested that a suitable ecological indicator should be (1) manageable, (2) it should be sensitive enough to slight variations of ambient stress, (3) it may show independence of control samples or reference values, (4) it must be applicable in broad geographical areas and in a range of possible communities and (5) must have relevance to policy and management. These characteristics are not easy to fulfill, especially in highly dynamic coastal environments subjected to multiple human-induced stressors (e.g., nutrients, contamination, temperature and CO_2 change), whose effects are still poor understood (Cloern 2001, Duarte et al. 2009). As the response of marine communities to coastal eutrophication is not merely dependent on nutrient concentrations (Bokn et al. 2003, Karez et al. 2004) but also the result of combined effects of variable nutrient regimes, biophysical factors (Nixon 1995, Schramm 1999, Nixon et al. 2001, Russell et

al. 2005, Gorman et al. 2009) and other sources of anthropogenic pressure such as pollutants, dissolved CO_2 and global temperature change (Cloern 2001, Wernberg et al. 2012), the variability in the response of macroalgal communities to enhanced nutrients may not always will imply a significant and direct increase in macroalgal indicators (Bokn et al. 2002, Bokn et al. 2003, Karez et al. 2004, Kraufvelin et al. 2006).

Macroalgae absorb and store nutrients for reproduction and growth, and so the levels of nutrient in the environment will have a direct effect on plant function. Macroalgae also play a crucial role in processing and removing nutrients from the environment, and so they can influence the eutrophication process. Bloom-forming macroalgae are well placed to respond to increases in nutrient concentrations as they have a range of opportunistic physiological traits (e.g. rapid growth and nutrient uptake rates and wide tolerance to environmental stress) that give them an advantage under such conditions (Fong et al. 1996, Kamer & Fong 2000, Lotze & Schramm 2000). However, the relationship between nutrient load, nuisance algae proliferation and changes in community composition is not easily defined, and a number of environmental feedback mechanisms such as the background ecology of marine communities and prevailing abiotic conditions will influence this relationship (Sand-Jensen & Borum 1991, Schramm 1999, Cloern 2001, Edgar et al. 2005, Macleod et al. 2007). Consequently, it is hard to attribute any scale or quantity to the appearance of these opportunistic algae before they “bloom” or to predict where and when such a change may take place. The effective prevention of impacts requires sensitive tools that can evaluate small variations in environmental stress indicated by cumulative changes in nutrient availability that could be overlooked due the unpredictability of ecosystem responses. Timely evaluation of impacts may be determined by the spatial variability and nature of nutrient loads, location-specific

environmental constraints, and the individual response of certain species (Schramm 1999, Cloern 2001, Tomasko et al. 2005)

4.1.1. Physiological responses of macroalgae as indicators of eutrophication

Many marine organisms respond to environmental changes at a physiological level before they show any visible evidence of change at a community level (Portner & Farrell 2008). Given the diversity of macroalgal species in temperate reef systems, it is likely that some species could serve as rapid response indicators on a scale of days to weeks (e.g., annual fast-growing species), whilst others (e.g., perennial species such as large brown algae) may respond more slowly, at seasonal and yearly scales. Since fast-growing and perennial macroalgae present different capabilities to store nutrients (Pedersen et al. 2010), this may be associated with the nutrient status of the system and the potential effect of combined natural and anthropogenic inputs (Wheeler & Bjornsater 1992, Schaffelke & Klumpp 1998). Experimental work in controlled systems has shown that both tissue nutrient levels and N:P ratios may increase when macroalgae are exposed to high nitrogen concentrations (Wheeler & Bjornsater 1992, Cohen & Fong 2006), where some fast-growing species can rapidly incorporate pulses of ammonia (Fujita 1985, McGlathery et al. 1996). However, nutrient values able to stimulate a biological response (e.g., opportunistic species abundance) generally must exceed thresholds concentrations to validate the use of such indicators and these levels may change when is escalated to field conditions. Relatively pristine ecosystems or low nutrient environments may show no effects in the short term, and this response has been documented in a number of studies (See Ruiz et al. 2001, Karez et al. 2004, Fourqurean et al. 2010). In these investigations biophysical factors other than nutrient availability (e.g., wave exposure) seemed to play critical roles in the control

of opportunistic' species (Kraufvelin et al. 2002, Kraufvelin 2007, Wernberg & Connell 2008, Kraufvelin et al. 2010). For this reason, monitoring not just the concentrations but also the effect of changes in nutrient concentrations under field conditions may be a more meaningful tool for prediction of overall impact and management. If physical gradients have the capacity to constrain and control ecological responses, similar circumstances may be expected with physiological processes such as nutrient uptake and incorporation. Shifts in species composition of opportunistic ephyphitic flora have been shown to precede seagrass loss, and this may be a more practical early warning sign for eutrophication (Cambridge et al. 2007). However, these changes in opportunistic abundances occurred late in the process of eutrophication and usually too close to the ecosystem turning point to be useful as management tools (Schramm 1999, Cambridge et al. 2007). Consequently, a major management concern regarding nutrient threshold exceedance is how to be proactive rather than reactive. This may require looking for subtler indicators of change and ecosystem response.

The chemical composition of macroalgae can provide a measure of nutrient load in the environment (Wheeler & Bjornsater 1992, Cohen & Fong 2006). The advantage of this over water column measurements directly is that the macroalgal samples would provide a temporally integrated evaluation of the nutrient load over time rather than just a measure of the conditions at a single point in time (Cohen & Fong 2006). Nutrient content has been shown to be a sensitive indicator of nutrient availability in seagrasses, indicating effect at a level when community indicators (e.g, ephyphitic species) seemed to be unresponsive to changes in nutrient availability (Fourqurean et al. 2010). The chemical composition of macroalgae has also been used to identify ecological and water quality effects of shrimp farms and Sewage Treatment Plants effluents (Lin & Fong 2008, Kim et al. 2014) (Cohen & Fong 2006).

Enhancement of nutrient availability can increase photosynthesis and hence plant biomass in macroalgae communities (Pedersen 1995). Hence, an alternative approach for assessing differences promoted by nutrient enrichment is to measure chlorophyll α fluorescence; this provides an estimation of photosynthetic performance (Maxwell & Johnson 2000). This approach uses an autonomous portable device, is non-destructive and easy to perform and would allow multiple specimens to be assessed in situ, (Parkhill et al. 2001, Harrington et al. 2005, Scherner et al. 2012, Scherner et al. 2013). The use of Rapid Light Curves (RLCs) and the derived parameters (F_v/F_m, α, E_k and rETR) can provide evidence about adaptive capabilities of plants under a diverse gradient of stress (Bilger et al. 1995, Maxwell & Johnson 2000, Scherner et al. 2012). For instance, experimental work on phytoplankton has determined that F_v/F_m remains high and independent of irradiance where nutrients are in excess, whereas it will be depressed where nutrients are limited (Kolber et al. 1988, Genty et al. 1989, Parkhill et al. 2001). Thus, when algal growth is not nutrient stressed, growth and photosynthesis is considered nutrient replete. Nutrient-replete conditions are expected in environments subjected to nutrient enrichment. Consequently, photosynthetic responses in macroalgae assessed throughout Chlorophyll-a fluorescence may be used as an indicator of nutrient enrichment. This method needs further field validation, because the photosynthetic responses in marine plants have been found to be highly variable, particularly due to light fields at surface and bottom and the potential for highly adapted tissue (Edwards & Kim 2010) It would be useful to measure the photosynthetic responses of macroalgae to a range of nutrient levels and across environmental gradients in order to establish dose-response relationships.

Effect of nutrient enrichment and environmental variables on physiological responses

Water motion, light, nutrient levels, salinity, and temperature are the fundamental factors that structure macroalgal communities and define their productivity (Hurd et al. 2014). These factors will influence absorption and assimilation rates, hence productivity. For example, experimental work has shown that the diffusion boundary layer (DBL), where nutrient incorporation takes place, is strongly influenced by water motion (Wheeler 1980, Hurd 2000). Consequently, any change in the DBL can affect the concentration of enzymes stimulated by nitrogen availability (Weissman 1972, Lobban & Harrison 1994). Thus nutrient assimilation can be enhanced/ constrained in individual plants through changes in the concentration of enzymes involved in nutrient sequestering, such as nitrate reductase and/or enzymes active during the process of photosynthesis (i.e., RuBisCO). On the other hand, changes in temperature can influence enzyme efficiency; with higher temperatures tending to decrease productivity (Lobban & Harrison 1994, Hurd et al. 2014).

Light level also has a major impact on macroalgal performance; light availability and quality (available wavelengths) changes naturally with depth and water clarity (Falkowski & Raven 1997) but this can be enhanced/ degraded by both changes in the natural environmental conditions (e.g. seasonal changes in river inputs/ turbidity, but also by the presence of anthropogenic pollutants). Salinity also plays a critical role in determining macroalgal performance, as changes in salinity will affect the internal osmotic gradients within the plants and therefore influence uptake rates (Lobban & Harrison 1994). Importantly, all of these environmental criteria interact, sometimes synergistically and sometimes antagonistically, such that the overall physiological response varies markedly in response to geographical, topographical and even seasonal differences in the prevailing environmental conditions (Hurd 2000, Denny 2006). Thus, the ultimate impact

of nutrification will differ depending on the species of macroalgae and microalgae present at any given time (Duarte 1995). Consequently, the ecological effects will be location specific.

4.1.2. Context-dependent responses to nutrient inputs

Field-based experiments at geographical scales are needed to clarify how location-specific differences in the effect of multiple factors may operate the level of impact in the whole system (Wernberg et al. 2012). This may provide insights about the capacity of a given ecosystem to cope with external stressors and the geographical/ environmental context for coherent ecosystem-based management. In addition, monitoring tools for rapid evaluation that may indicate subtle alterations of nutrient supplies at relevant spatial scales are also needed.

It is important to improve our understanding of the combined influence of both environmental factors and nutrient enrichment, and to establish how these might affect the physiological responses of macroalgae. This information could have significant implications for coastal planning, particularly in a rapidly changing environment where baselines studies are often non-existent. If it was possible to identify physiological changes in response to low or moderate levels of nutrient enrichment, this could provide an early indicator of nutrient stress that could be used to trigger a management response well before any major changes in community structure occur. This study aimed to determine if physiological characteristics (chemical/nutrient composition and photosynthetic performance) of coexistent key macroalgal species (*Sargassum fallax*, *Ecklonia radiata* and *Ulva* sp.) responded to experimental nutrient additions in an estuarine system, and how this response might change along a natural

environmental gradient. This approach allows evaluation of the extent to which both nutrient and environmental variability might amplify or constrain the observed physiological responses to nutrient enrichment.

4.2. Methods

4.2.1. Study sites and collection of samples

All samples were collected on three shallow rocky reefs (1.5 - 2.5 m depth) at the D'Entrecasteaux Channel and lower reaches of the Huon Estuary in SE Tasmania (see Figure 1 Chapter II). Tinderbox at the upper Channel, Green Island in the mid part of the Channel and Ninepin Pt. in the lower reaches of the Huon estuary as described in earlier chapters. The D'Entrecasteaux Channel is exposed to a mix of dissolved and particulated anthropogenic nutrients principally from salmon farming activities (27,00 tonnes/year) and effluents from nine wastewater treatment plants (see Figure 1, Chapter II). Catchment inputs from agriculture and forestry also constitute a historical source of exogenous nutrients (Ross & Macleod 2013).

4.2.2. Algal tissue

Five independent tissue samples of the fucoid *Sargassum fallax*, the Laminaria *Ecklonia* radiata, and the fast-growing green algae *Ulva* spp. were (Figure 4.1) taken from every treatment and control plot displayed at every location as described earlier on Chapter II. However, *E. radiata* was not found in the experimental plots during spring and *Ulva* spp. was not found during both seasons in Ninepin Pt. The tissue samples were transported in an ice chest to the laboratory and gently scrubbed with a soft brush to remove any epibionts and then rinsed with abundant fresh water to remove $NaSO_4$ and other seawater salt remnants. The samples were oven air-dried at

60°C during 24 hours and then grounded to a fine powder. Between 0.013 and 0.014 mg of the powder was weighed in a digital balance and the record weight was used for calculations. Nitrogen content was determined with an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., UK). The total phosphorus determination was undertaken with the alkaline persulphate digestion method. Approximately 100 - 200 mg dried and grounded solid samples (record weight used for calculations) were mixed with 20mL of MQ water (Ultrapure' water of "Type 1") and 10mL of the oxidizing solution in 50mL autoclaved tubes and then analyzed using a colourimetric FIA method (Hosomi & Sudo 1986). Values in mg g^{-1} of sample were transformed to μmol g^{-1} dried tissue. To determine potential differences in macroalgal nutrient limitation the C:N:P molar ratio was calculated simply as the quotient between the total molar content (μmol/g sample) of C, N and P of each sample

4.2.3. Calculations of nutrient content and C:N:P ratios

The nutrient content of algal tissue was calculated as follows:

- Nutrient content = Mg of Nutrient × Sample weight (g) = Mg/g of nutrient per g of sample.

The C:N:P Molecular ratio was calculated as:

- Elemental nutrient content = mg/g samples × Molecular weight of nutrient (i.e., Carbon = 12.017; Nitrogen = 14.006; Phosphorus = 30.97)

4.2.4. Water column

The nutrient concentration in the water column was determined as in Chapter 2. Where samples (n=3) were collected 10 cm and 1m away from the dispenser bags in the treatment plots and randomly in the control plots. The calculation of N:P ratios was performed to determine water nutrient limitation between locations and if this coincided in different seasons. N and P availability in the water column was contrasted with the Redfield ratio N:P = 16. This ratio reflects a general trend of the elemental composition of plankton and the nutrients composition in the surrounding seawater (Redfield 1958, Atkinson & Smith 1983, Wheeler & Bjornsater 1992).

4.2.5. Statistical analysis

Univariate

The nutrient composition of macroalgae under enriched and control conditions across locations were conducted with a mixed model ANOVA as described above for community composition. As specific nutrient depletion is expected to occur on a seasonal base at temperate reefs during spring/summer (Hurd et al. 2014) the analyses were conducted separately for each season. The null hypothesis tested was that the effect of the nutrient additions (treatment conditions) caused no differences in the tissue nutrient composition between natural and control specimens and throughout the locations. When differences were not detected between seasons and nutrient addition, to explore potential differences dependant of location t-independent samples test were undertaken to compare differences between two locations. Analyses were undertaken with SPSS 2.1©(IBM® Statistics, 2012).

4.2.6. Photosynthetic responses

The photosynthetic performance of *Sargassum fallax*, *Ecklonia radiata* and *Ulva* spp. was evaluated in Tinderbox and Green Island, while due to the absence of Ulva specimens during the sampling campaign at Ninepin Point, only *S. fallax* and *E. radiata*, were assessed at this location. The evaluation was carried out in situ during winter, spring and summer of 2012. The specimens were monitored across seasons approximately every three months.

In order to evaluate shade-adapted samples (avoiding highly variable light fields at surface usually influenced by wave action, shading by clouds, tidal regimes and solar oscillation [Falkowski, 1990]), the samples were obtained from the middle part of the plant in *S. fallax*, while *E. radiata* samples were taken randomly. *Ulva* spp. samples were taken at the same depth of brown algae when was possible. All the samples from artificially enriched areas were taken within a radius of ~1 m around the dispenser bags. The samples from natural areas were taken randomly across the sampling plots approximately at the same depth.

The photosynthetic performance was evaluated through measurements of chlorophyll-a fluorescence using a pulse of amplitude-modulated fluorometer (Diving-PAM Walz GmbH, Effeltrich, Germany, 1998). Rapid light curves (from now on referred as RLCs) were performed on five samples of photosynthetic tissue of each species from each experimental area across all sites. Sets of five samples were collected each time and set in clip-shaded adaptors, which hold and shade the sample for their assessment. Each sample was acclimatized 15 minutes in darkness, to ensure the complete opening of the photosystems prior to direct assessment at in situ conditions.

4.2.6.1 Rapid Light Curves

The diving-PAM used here for in-situ sampling was equipped with an internal halogen lamp, which provides actinic illumination for each step of the RLC, as well as the saturating pulses. The light pulses were calibrated against a Li-Cor quantum sensor (Li-Cor, Lincoln, NE, USA). RLCs were measured using the PAM's software routine, where the light pulses are incremented in eight 'light steps' (0, 4, 14, 29, 52, 78, 108, 162, 224 µmol $m^{-2}s^{-1}$). Light Curve-Intensity (LC-INT) sets the range of irradiance provided by the RLC. As the relative electron transport rate (rETR) always reaches a plateau in presence of saturating light (It may decline during the last couple of light steps), to ensure optimal fluorescence detection by the fluorometer, the configuration was previously evaluated and set as follows: for shade-adapted samples Gain = 2, LC-INT = 1; for light adapted samples, Gain = 2 and LC-INT = 2 to ensure saturation (Ralph & Gademann 2005, Scherner et al. 2012). Additionally, the Auto-zero function was carried out before each RLC measurement to reduce false signals originated by internal 'pick ups' of the system and traces of scattered measured light that may reach the photodetector (Handbook of operation).

Statistical analysis

Fluorescence data was exported to Sigma Plot 11.0 (Systat Software Inc., 2008). As the relative electronic transport rate (rETR) is a function of the irradiance, the plots usually show a classical shape with a sustained rise until they reach a plateau where the photosynthesis theoretically became limited (Ralph & Gademann 2005). Therefore, light steps were plotted against the relative ETR and then the curves were fit to an exponential decay function with two parameters (Platt et al. 1980) to obtain the $rETR_{max}$ using a simplification of the Marquardt-Levenberg regression algorithm:

$rETR = ETR_{max}*(1-e^{(-\alpha*I/rETR_{max})})$

Where $rETR_{max}$ is the photosynthetic capacity at saturating irradiance, α corresponds to the initial slope of the curve before reach the plateau, which represents the efficiency in light utilisation. The light saturating point coefficient, E_k, is the ratio between ETR_{max} and α. The quantum yield value (or "efficiency" of photosynthesis), F_v/F_m, was obtained directly from the PAM fluorometer according Saroussi & Beer (Saroussi & Beer 2007, Beer et al. 2014). The univariate analysis of photosynthetic parameter of the different macroalgal species was tested as referred above for nutrient composition. To compare RLCs from treatment and control specimens one-way ANOVAs were undertaken for each location and season separately comparing each light step response (rETR values) within each replicated curve (n = 5).

4.2.7. Relationship between physiological responses and environmental variables

Prevailing environmental variables, light, wave exposure, salinity, temperature and nutrient loads were assessed as referred in Chapter 2. To analyse and model relationships between the physiological responses (i.e., chemical composition and photosynthetic performance) and abiotic variables a distance based linear model based on Euclidean similarity (DISTLM) was undertaken. Environmental data was normalised to adjusting the differences in the scale of the different variables. Akaike's selection criterion was used to evaluate the model. The responses in nitrogen content and N:P ratios, as well as, the photosynthetic parameters (F_v/F_m, ETR_{max} and E_k) and the influence of abiotic variables were plotted using distance based Redundancy Analysis

(Legendre & Anderson 1999). Colinearity of the abiotic data was assessed via Draftsman plots, and where high levels of colinearity were detected (e.g., $|r| \geq 0.95$) variables were arbitrarily removed from the analysis. All multiparametric analyses were undertaken using PRIMER 6.0© (2006).

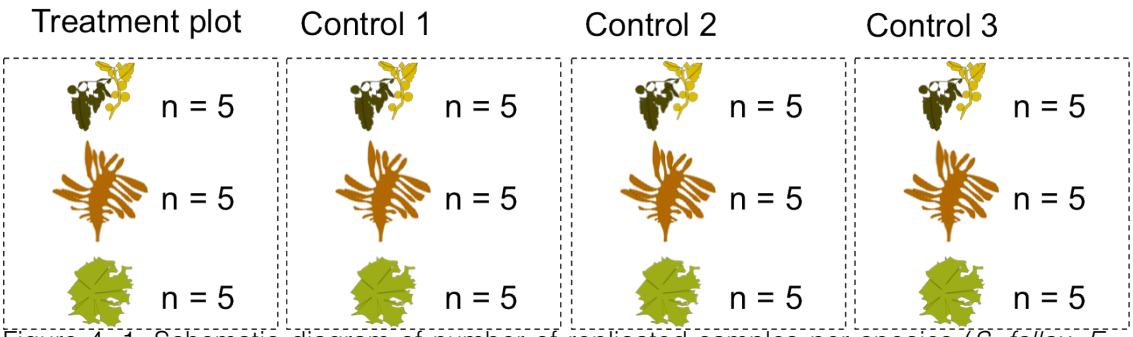

Figure 4. 1. Schematic diagram of number of replicated samples per species (*S. fallax*, *E. radiata* and *Ulva* spp.). This sampling design was repeated at each location in spring and summer. T=Treatment site, C1, 2, 3 = Control sites.

4.3. Results

4.3.1. Nutrient content in algal tissue

Sargassum fallax

Sargassum fallax displayed no significant increases in Nitrogen-tissue content (from now on referred as N-content) in treatment plots across locations during spring. However, during summer N-content from treatment sites were significantly different between locations (Table 1A). Treatment plants at Green Island and Ninepin Pt. showed increases in N-tissue content over a 50% compared with specimens from control plots (Figure 6 A). Whilst N-content in Tinderbox appeared elevated in both treatment and control specimens (Figure 6 A). The Phosphorus-tissue content (from now on referred as P-content) was not consistently affected by nutrient additions at any location. However, it did differ significantly between locations in summer (Table 4.1 B); with the specimens from Tinderbox being 80% higher than other locations (Figure 4.2 B). Carbon-tissue content was not significantly enhanced by the nutrient additions at any location during either spring and summer. However, C-tissue levels differed significantly between locations in summer (Table 1 C). Specimens from Tinderbox (Both in treatment and control sites) showed 10% lower C-tissue levels than plants from elsewhere (Figure 6 C). However, C-levels remained fairly constant between both seasons and locations. N:P ratios did not show a treatment effect but differed between locations in summer (Table 4.1 D). N:P ratios ranged between 35-60, indicating higher N loads compared to P. This was observed across all locations excepting at Tinderbox in summer (Figure 4.2 D) where P-content

showed elevated levels. The highest mean N:P ratios were observed over summer at Green Island and Ninepin Pt., suggesting greater N-tissue availability at these sites at this time, however, interestingly during the same period at Tinderbox the N:P ratio was at its lowest (N:P = 18. Figure 4.2 D). The effect of nutrient addition on the C:N ratios varied significantly between locations during summer (Table 1E). Specimens from treatment plots usually experienced C:N ratios < 15 in both seasons and only at Tinderbox the plants from control sites showed similar values (Figure 6 E). Instead, control specimens from Green Island and Ninepin Pt. showed significantly higher C:N ratios (80% and 65%, respectively) than specimens from treatment conditions (Figure 6 E).

Ecklonia radiata

Similarly, the effect of nutrient additions on N-tissue levels of *E. radiata* varied significantly across locations over summer (Table 1 B). As noted with *S. fallax*, N-content values were equally high in specimens from both treatment and control plots over summer. However, unlike *S. fallax*, N-tissue levels in Ninepin Point during summer presented no differences. Green Island specimens from treatment conditions showed approximately 50% higher N-tissue levels than control specimens over summer (Figure 7 A). P-content showed a similar effect of location during summer (Table 4.1 B) with around 50% higher levels at Tinderbox than at other locations (Figure 4.3 B). C-tissue levels showed no significant effects in spring and summer (Table 1 C), and were characterised by higher variability compared with *S. fallax* (Figure 7 C). N:P ratio varied between 12 and 35 (Figure 4 D) and was generally similar across seasons and locations, with no detectable effect of nutrient addition (Table 4.1 D), which suggest a range of variation from more to less P availability respect N in the tissue composition. On the other hand, C:N ratios

were generally > 15 in all locations during spring (Figure 7 E). Conversely, during summer, specimens from Tinderbox exhibited values ~ 15, while control specimens from Green Island and Ninepin Pt. (C:N ratios > 25) showed C:N ratios 48% and 16% higher than treatment specimens (Figure 7 E).

Ulva spp.

Ulva specimens showed no significant treatment effects on N-content in either spring or summer (Table 1 C), but there were patterns of difference between locations and seasons. N-content was in average 53% higher at treatment plots relative to controls at Tinderbox during spring. Also, N-levels showed being 40% higher at treatment conditions at Green Island in summer (Figure 8 A). C-tissue levels presented homogeneous values (between 20-25) across location in both seasons (Figure 8 C) and showed no significant response to treatment conditions (Table 1 C). There was no treatment or location effect on the P-content of *Ulva* (Table 4.1 B). However, the results suggest that P-content varied between locations in spring showing an average of 46% greater P-content in specimens from treatment plots compared with control specimens from Tinderbox. Whilst specimens from Green Island showed relatively high P-content in specimens from all experimental plots during this period (Figure 4.4 B). In summer, P-content remained similar in both locations and treatment sites. N:P ratios showed no statistically significant response to treatment conditions and no effect of location (Table 4.1 D). The N:P ratios in specimens from Tinderbox during spring were relatively stable (N:P ratios 30-39) but there was greater small-scale spatial variability in nutrient content during summer (N:P ratios ~20-45; Figure 4.4 D).). C:N ratios in *Ulva* showed that with the exception of Green Island in spring, all specimens from treatment conditions presented C:N

ratios < 15 during both seasons (Figure 8 E). However, treatment specimens presented no significant differences respect to control specimens at any location.

Table 4. 1. Summary of Mixed-model ANOVA comparing the effect of nutrient additions on (A) nitrogen, (B) Phosphorus, (C) Carbon tissue content and the resulting (D) N:P and (E) C;N ratios in *S. fallax*, *E. radiata* and *Ulva* spp. within three reefs in The D'Entrecasteaux Channel during spring and summer. (**) Indicates $p < 0.01$, (*) indicates $p < 0.05$.

	Sargassum fallax				*Ecklonia radiata*				*Ulva* spp.			
(A) Nitrogen	Spring		Summer		Spring		Summer		Spring		Summer	
	F	df	F	df	F	df	F	df	F	df	F	df
Treatment	0.2	1	3.6	1	2.7	1	0.9	1	0.3	1	1.5	1
Location	1.5	2	1.1	2	0.1	2	1.8	2	0.8	2	7	2
T × L	3	2	7.1*	2	0.9	2	16*	2	6.7	2	3.4	2
Plot (Treatment)	0.4	2	0.3	2	0.4	2	0.2	2	1.4	2	0.1	2
Location×Plot (Treatment)	1.7	4	8**	4	2.7	4	1.8	4	2.2	4	1.7	4
(B) Phosphorus	Spring		Summer		Spring		Summer		Spring		Summer	
	F	df	F	df	F	df	F	df	F	df	F	df
Treatment	0.1	1	0.6	1	0.6	1	1.2	1	0.7	1	---	1
Location	1.8	2	98*	2	4.5	2	24*	2	0.4	2	79	2
T × L	2.1	2	1.5	2	3.2	2	2.7	2	56*	2	0.2	2
Plot (Treatment)	0.3	2	1.4	2	2.5	2	4.3	2	1	2	0.08	2
Location×Plot (Treatment)	2.4	4	2.2	4	3.4	4	1.7	4	2	4	2.3	4
(C) Carbon	Spring		Summer		Spring		Spring		Summer		Spring	
	F	df	F	F	df	F	F	df	F	F	df	F
Treatment	0.2	1	0.2	0.2	1	0.2	0.2	1	0.2	0.2	1	0.2
Location	1.4	2	15*	1.4	2	15*	1.4	2	15*	1.4	2	15*
T × L	1	2	0.7	1	2	0.7	1	2	0.7	1	2	0.7
Plot (Treatment)	1.5	2	0.7	1.5	2	0.7	1.5	2	0.7	1.5	2	0.7
Location×Plot (Treatment)	1	4	2.1	1	4	2.1	1	4	2.1	1	4	2.1
(D) N:P ratio	Spring		Summer		Spring		Spring		Summer		Spring	
	F	df	F	F	df	F	F	df	F	F	df	F
Treatment	1.2	1	4.3	1.2	1	4.3	1.2	1	4.3	1.2	1	4.3
Location	0.03	2	69*	0.03	2	69*	0.03	2	69*	0.03	2	69*
T × L	1.2	2	1	1.2	2	1	1.2	2	1	1.2	2	1
Plot (Treatment)	2.3	2	0.7	2.3	2	0.7	2.3	2	0.7	2.3	2	0.7
Location×Plot (Treatment)	1	4	2.4	1	4	2.4	1	4	2.4	1	4	2.4
(E) C:N ratio	Spring		Summer		Spring		Summer		Spring		Summer	
Treatment	0.2	1	4	1	1.3	1	1.2	1	1.4	1	1.4	1
Location	2	2	2.5	2	3	2	5	2	0.9	2	9.4	2
T × L	5.1	2	6.1*	2	2.6	2	13*	2	10	2	6.2	2
Plot (Treatment)	0.6	2	0.3	2	0.6	2	4	2	2.4	2	0.07	2
Location×Plot (Treatment)	1.9	4	9.2**	4	1.4	4	1.1	4	2.4	2	1.5	4

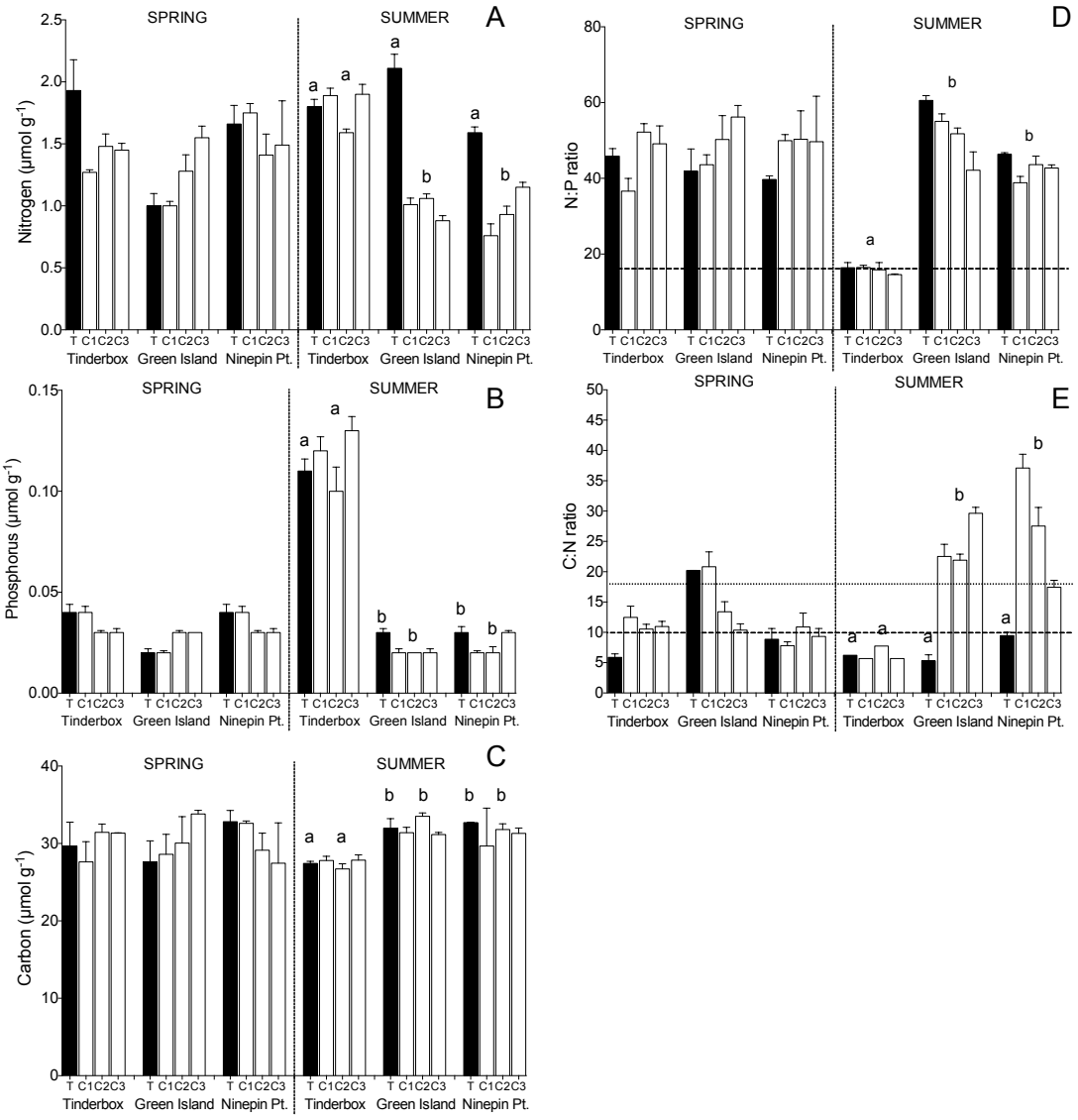

Figure 4. 2. Tissue content of (A) Nitrogen, (B) Phosphorus and (C) molar N:P ratios of *S. fallax* (Mean ± SE) during spring and summer at Tinderbox, Green Island and Ninepin Point. Solid bars show the Treatment plots; Clear bars show control plots. Letters show significant differences between locations (HSD Tuckey). Dashed line in E indicates critical values for N-limiting tissue content according D'Elia & DeBoer (1978) and doted line illustrates critical values according Atkinson & Smith (1983).

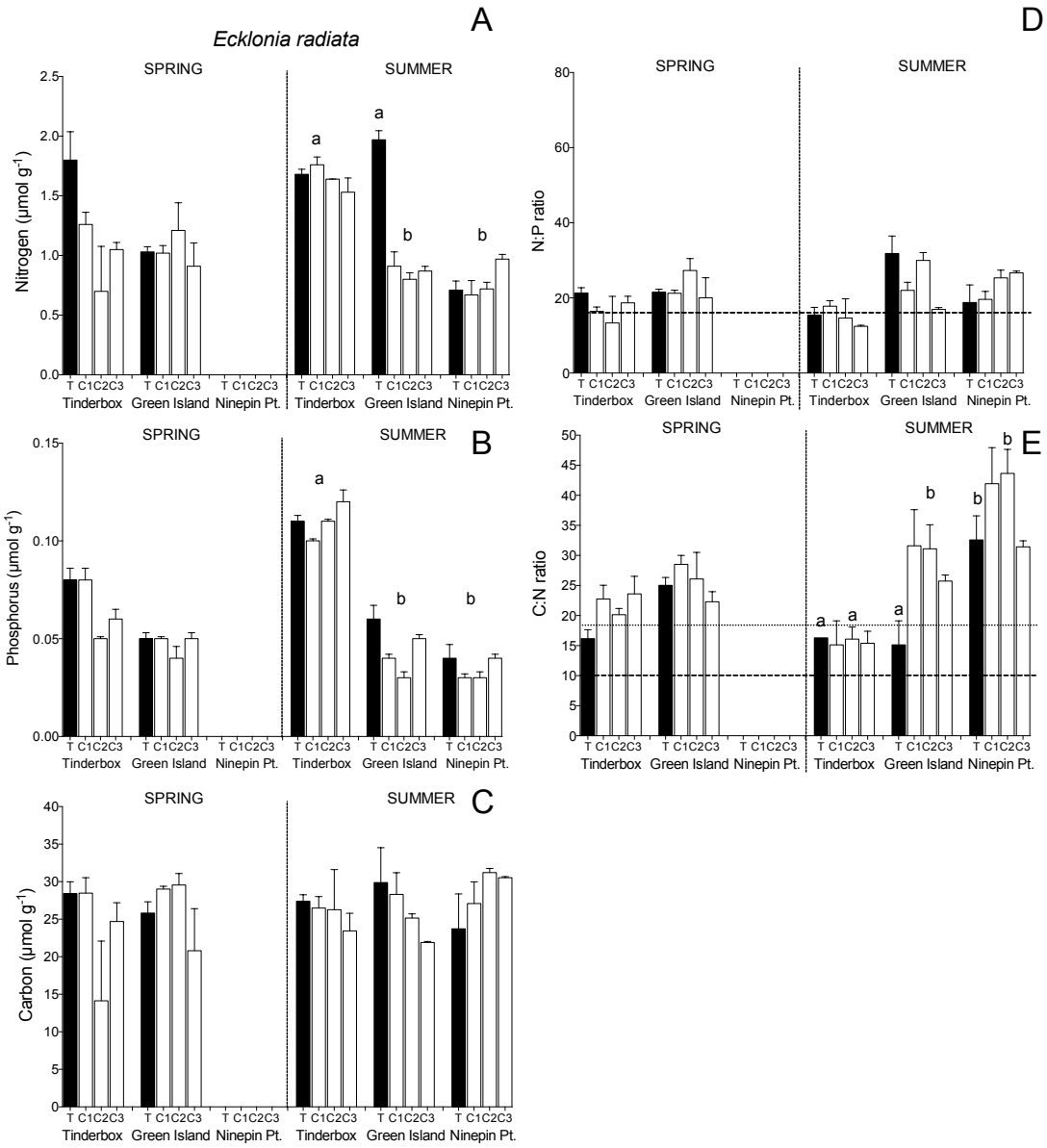

Figure 4. 3. Tissue content of (A) Nitrogen, (B) Phosphorus and (C) molar N:P ratios of *E. radiata* (Mean ± SE) during spring and summer at Tinderbox, Green Island and Ninepin Point. Solid bars show the Treatment plots; Clear bars show control plots. Letters show significant differences in the variation of treatment effects across locations (HSD Tuckey). Dashed line in E indicates critical values for N-limiting tissue content according D'Elia & DeBoer (1978) and doted line illustrates critical values according Atkinson & Smith (1983).

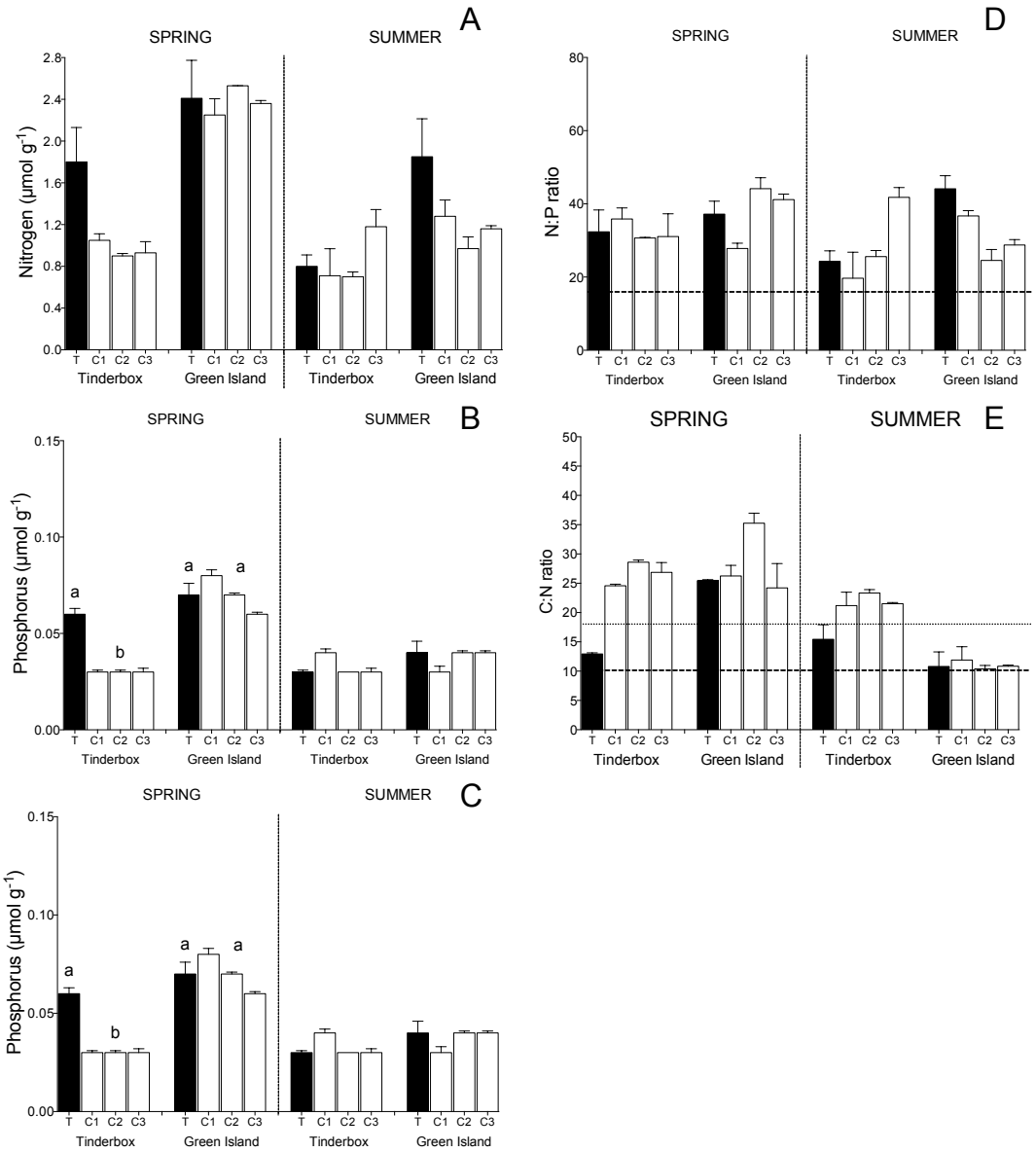

Figure 4. 4. Tissue content of (A) Nitrogen, (B) Phosphorus and (C) molar N:P ratios of *Ulva* spp. (Mean ± SE) during spring and summer at Tinderbox, Green Island. Solid bars show the Treatment plots; Clear bars show control plots. Letters show significant differences in the variation of treatment effects across locations (HSD Tuckey). Dashed line in E indicates critical values for N-limiting tissue content according D'Elia & DeBoer (1978) and doted line illustrates critical values according Atkinson & Smith (1983).

4.3.2. Water column nutrient concentrations

In the present study, available nitrogen in control conditions during spring and summer (0.07 – 0.6 μM l^{-1}) were similar to background levels observed in previous environmental evaluations for the study region (Ross & Macleod 2013). Phosphorus (0.1 – 0.3 μM l^{-1}) also displayed similar concentrations to those from previous surveys. In contrast, total nutrient levels next the experimental sources reached values between 3.2 – 21 μM l^{-1} of available nitrogen and 0.3 – 3.9 μM l^{-1} of reactive phosphorus. Interestingly, these concentrations showed a substantial reduction 1 m from the nutrient supply units and although averages appeared generally above ambient levels high variability among data meant that there were no significant differences with ambient concentrations (Table 4.2). Overall, ambient nutrients presented a marked seasonal pattern with maximal values in winter decreasing to spring and summer evidencing the effect of background nutrient concentrations (Table 4.2). Values decreased during spring at 1m but still remained close to ambient levels, this trend was clearer with NO_x (Table 4.2) for both Tinderbox and Green Island. Summer presented slightly higher values, however, the results showed that nutrient concentrations were subjected to greater variation both between and within locations.

4.3.2.1. Nutrient ratio in the water column

N:P ratios in the water column varied widely, showing differences between treatments, locations and seasons (F 3.4; df 8; $p < 0.01$). Interestingly, a divergent pattern between locations was observed (Figure 5). However, a clear response to nutrient additions was difficult to establish due to high variation, especially at Green Island. Average N:P ratios in control plots remained between 1 and 4 at Tinderbox and Ninepin Point, indicating more P

than N, whilst, N:P ratios from Green Island fluctuated between 0.6 and 9.3 (less N-limitation). When N:P ratios were examined within particular locations, Tinderbox exhibited significant variations between distances to the source and seasons (F 2.9; df 4; p = 0.04). The post hoc analysis showed that in summer N:P ratios close to the nutrient source (10 cm) were significantly higher (less N-limited) than samples from control plots (Figure 5 A), whilst, a clear seasonal difference was only observed at Ninepin Pt. (F 3.5; df 4; p = 0.02; Figure 5 C). During summer Green Island showed wide between-plot variability (Figure 5 B), which obscured any potential significant difference between treatment effects and season (F 2; df 4; p = 0.1).

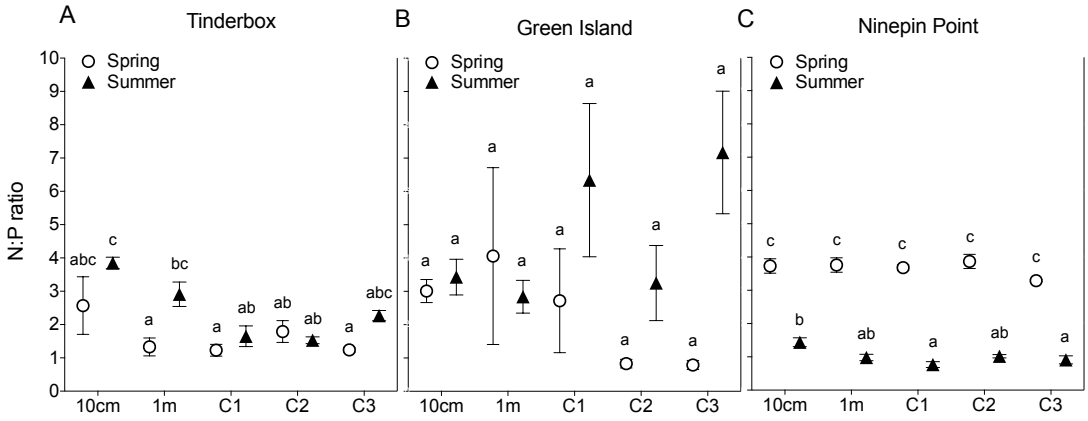

Figure 4. 5. Water column N:P ratios (Mean ± SE) at (A) Tinderbox, (B) Green Island and (C) Ninepin Pt. (n=3) at three distances from the nutrient source (1cm;1m >50m). Letters show significant differences between seasons and distances for individual locations (HSD Tuckey).

Table 4. 2. Water column Ammonia, Fraction of Reactive Phosphorus (here Phosphorus) and NOx concentrations (mean μM l^{-1} ± SD) at three distances from experimental nutrient sources for each location from pre-treatment conditions (Autumn). (*) and numbers in bold indicates significant differences (two-way ANOVA).

A	Ammonia	Autumn	Winter	Spring	Summer
Tinderbox	Ambient	0.3 ± 0.1	0.8 ± 0.01	0.2 ± 0.1	0.3 ± 0.1
	1m	0.35 ± 0.06	0.9 ± 0.4	0.3 ± 0.1	0.6 ± 0.1
	10 cm	---	**6 ± 4***	**1.6 ± 0.6***	**4.3 ± 1.5***
Green Island	Ambient	0.29 ± 0.3	0.3 ± 0.06	0.35 ± 0.4	0.6 ± 0.4
	1m	0.2 ± 0.3	0.9 ± 0.3	1.1 ± 1.3	0.7 ± 0.3
	10 cm	---	**2.0 ± 0.1***	**1.6 ± 0.6***	**3.9 ± 2.8***
Ninepin Pt.	Ambient	0.3 ± 0.06	0.45 ± 0.1	0.4 ± 0.06	0.06 ± 0.06
	1m	0. 5 ± 0.3	0.9 ± 0.4	0.5 ± 0.1	0.5 ± 0.3
	10 cm	---	**9.9 ± 11***	0.6 ± 0.2	**7.3 ± 0.8***
B	Phosphorus	Autumn	Winter	Spring	Summer
Tinderbox	Ambient	0.2 ± 0.03	0.5 ± 0.5	0.3 ± 0.001	0.2 ± 0.03
	1m	0.2 ± 0.02	0.3 ± 0.05	0.3 ± 0.003	0.3 ± 0.003
	10 cm	---	1.3 ± 0.8	0.8 ± 0.1	**1.3 ± 0.4***
Green Island	Ambient	0.2 ± 0.03	0.3 ± 0.01	0.3 ± 0.001	0.1 ± 0.03
	1m	0.2 ± 0.003	0.4 ± 0.06	0.3 ± 0.03	0.3 ± 0.06
	10 cm	---	**0.5 ± 0.9***	**0.65 ± 0.1***	**1.3 ± 0.8***
Ninepin Pt.	Ambient	0.1 ± 0.02	0.3 ± 0.03	0.3 ± 0.2	0.1 ± 0.01
	1m	0.14 ± 0.02	0.4 ± 0.06	0.4 ± 0.3	0.2 ± 0.01
	10 cm	---	**1.9 ± 1.9***	0.4 ± 0.05	**2.2 ± 2.1***
C	NOx	Autumn	Winter	Spring	Summer
Tinderbox	Ambient	0.1 ± 0.03	0.6 ± 0.03	0.1 ± 0.02	0.1 ± 0.03
	1m	0.06 ± 0.02	0.6 ± 0.04	0.1 ± 0.001	0.1 ± 0.03
	10 cm	---	1.7 ± 0.7	0.5 ± 0.1	**0.9 ± 0.3***
Green Island	Ambient	0.05 ± 0.01	0.7 ± 0.05	0.03 ± 0.02	0.05 ± 0.02
	1 m	0.06 ± 0.02	0.8 ± 0.08	0.05 ± 0.03	0.2 ± 0.1
	10 cm	----	**1 ± 0.02***	**0.4 ± 0.1***	**0.9 ± 0.6***
Ninepin Pt.	Ambient	0.08 ± 0.01	0.8 ± 0.02	0.9 ± 0.06	0.01± 0.01
	1m	0.1 ± 0.05	1.0 ± 0.08	0.95 ± 0.02	0.2 ± 0.1
	10 cm	----	**3.0 ± 2.6***	0.9 ± 0.09	**1.8 ± 1.7***

4.3.3. Photosynthetic response to nutrient enrichment

The photosynthetic parameters (F_v/F_m, ETR_{max} and E_k) for *S. fallax*, *E. radiata* and *Ulva* spp. showed no significant differences between treatment and control sites, or between locations during spring/ summer (Table 4.3 A, B and; Figure 4.6, 4.7 and 4.8 A, B and C respectively). These responses were presumably limited by high levels of variation in specimens from different plots and by the relative small increase in nutrient concentrations at 1m from the supply units.

4.3.3.1. Rapid Light Curves (RLCs)

RLCs show a range of location-specific responses to season and treatment, but again there were no consistent patterns across locations. There were differences in the physiological responses at treatment conditions in Green Island and Ninepin Pt. during summer for *Sargassum fallax*. *S. fallax* showed an increase of approximately 30% in the relative electron transport rate (rETR) under treatment conditions relative to control specimens during summer at Green Island ($F = 5.9$; df 1; $p = 0.01$, Figure 4.9) and Ninepin Pt. ($F = 4.9$; df 1; $p = 0.02$). *Ecklonia radiata* showed a similar response to nutrient additions at Green Island in spring ($F = 11.5$; df 1; $p < 0.05$) and summer ($F = 4.8$; df 1; $p = 0.03$; Figure 4.10), while at Ninepin Pt. the response to treatment conditions was significant during spring only ($F = 39$; df 1; $p < 0.01$; Figure 4.10). RLCs for *Ulva* spp. showed no clear saturating patterns of saturation and suggested that the physiological response was the same between treatment conditions and locations (Figure 4.11).

Table 4. 3. Summary of Mixed model ANOVA comparing the effect of nutrient additions on photosynthetic efficiency (F_v/F_m), maximal electron transport rate (ETR_{max}) and light saturation (E_k) in (A) *Sargassum fallax*, (B) *Ecklonia radiata* and (C) *Ulva* spp. within three different reef systems during spring and summer throughout the D'Entrecasteaux Channel. (*) Indicates $p < 0.05$; (**) Indicates $p < 0.01$.

	Spring			Summer		
A. *Sargassum fallax*	F_v/F_m	ETR_{max}	E_k	F_v/F_m	ETR_{max}	E_k
	F-ratio	F-ratio	F-ratio	F-ratio	F-ratio	F-ratio
Treatment	0.9	---	---	1.5	0.2	0.2
Location	1	---	---	0.5	0.6	0.4
T × L	3.7	0.1	0.1	3.1	10*	12*
Plot (Treatment)	1.5	0.5	0.3	0.2	3.1	2.4
Location×Plot (Treatment)	0.8	29.7**	33.7**	1.7	0.4	0.4
	Spring			Summer		
B. *Ecklonia radiata*	F_v/F_m	ETR_{max}	E_k	F_v/F_m	ETR_{max}	E_k
	F-ratio	F-ratio	F-ratio	F-ratio	F-ratio	F-ratio
Treatment	3.2	1.4	1.4	---	2.7	2.2
Location	0.4	2.1	2.2	---	0.9	0.6
T × L	0.7	9	8.8	0.2	16*	128
Plot (Treatment)	0.5	1	1.2	0.8	6	6*
Location×Plot (Treatment)	4.8**	3.1*	3.1*	10**	0.4	0.1
	Spring			Summer		
C. *Ulva* spp.	F_v/F_m	ETR_{max}	E_k	F_v/F_m	ETR_{max}	E_k
	F-ratio	F-ratio	F-ratio	F-ratio	F-ratio	F-ratio
Treatment	---	2.5	2.8	7	15.7	---
Location	1	20	276	---	4	4.5
T × L	0.1	1.8	0.7	0.2	0.5	0.3
Plot (Treatment)	0.2	0.7	7.7	1.8	0.6	0.5
Location×Plot (Treatment)	18.7**	1	0.2	0.4	29**	34**

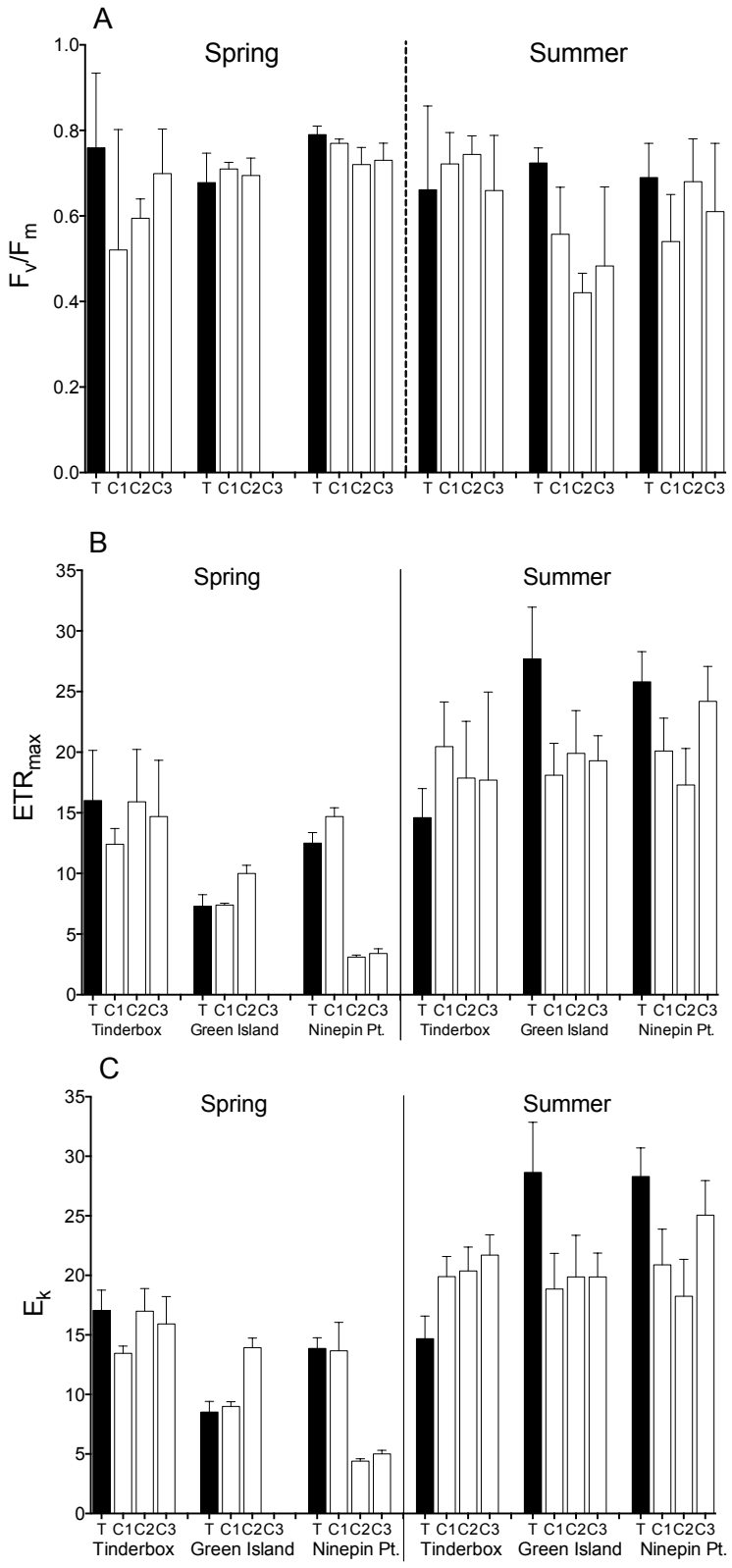

Figure 4. 6. Photosynthetic parameters (A) F_v/F_m, (B) ETR_{max}, (C) and E_k (Mean ± SE) in specimens of Sargassum fallax at three shallow rocky reefs in The D'Entrecasteaux Channel. Black bars showed responses in algae exposed to experimental nutrient additions (T = Treatment plots) and Ambient conditions (C1, C2, C3 = Control plots).

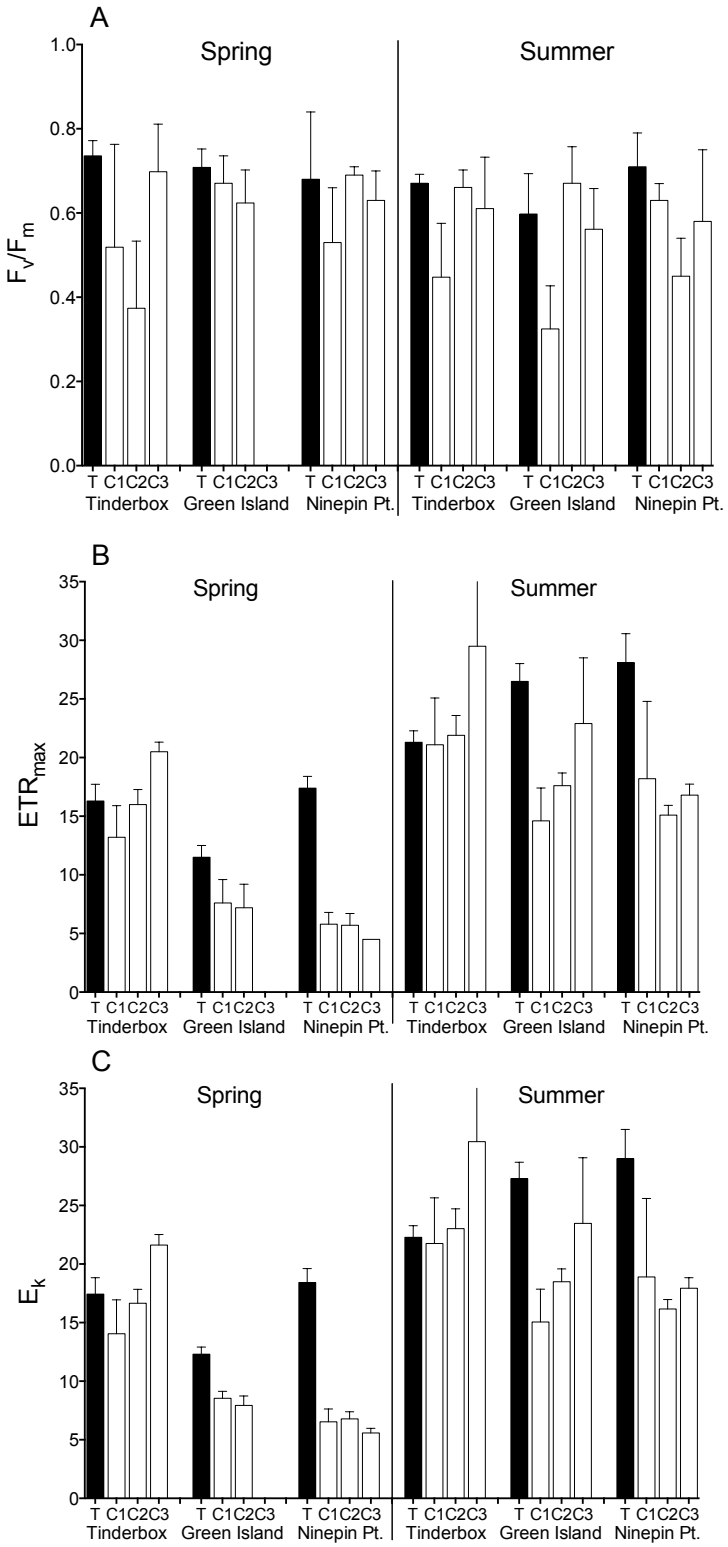

Figure 4. 7. Photosynthetic parameters (A) F_v/F_m, (B) ETR_{max}, (C) and E_k (Mean ± SE) in specimens of Ecklonia radiata at three shallow rocky reefs in The D'Entrecasteaux Channel. Black bars showed responses in algae exposed to experimental nutrient additions (T = Treatment plots) and Ambient conditions (C1, C2, C3 = Control plots).

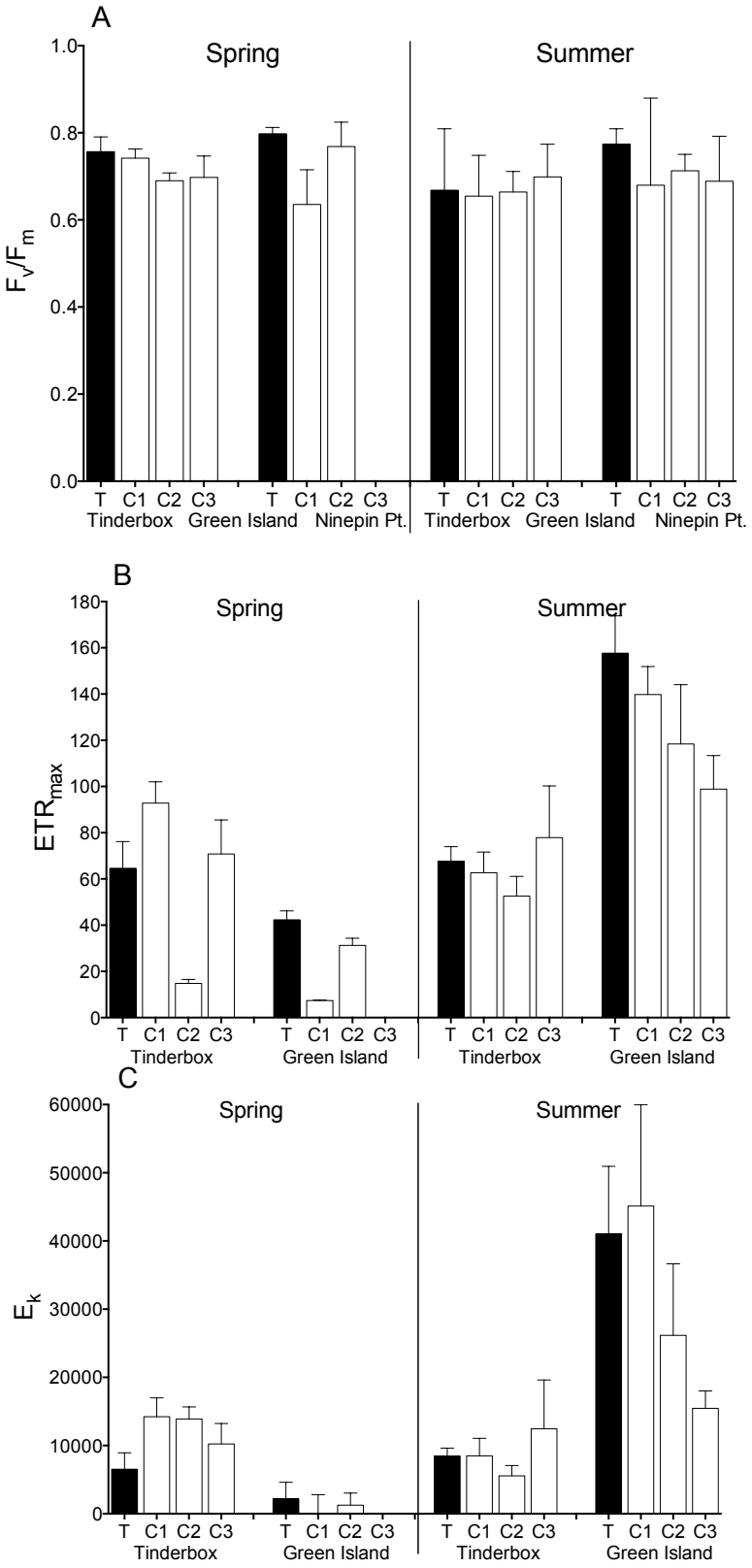

Figure 4. 8. Photosynthetic parameters (A) F_v/F_m, (B) ETR_{max}, (C) and E_k (Mean ± SE) in specimens of *Ulva* spp. at three shallow rocky reefs in The D'Entrecasteaux Channel. Black bars showed responses in algae exposed to experimental nutrient additions (T = Treatment plots) and Ambient conditions (C1, C2, C3 = Control plots).

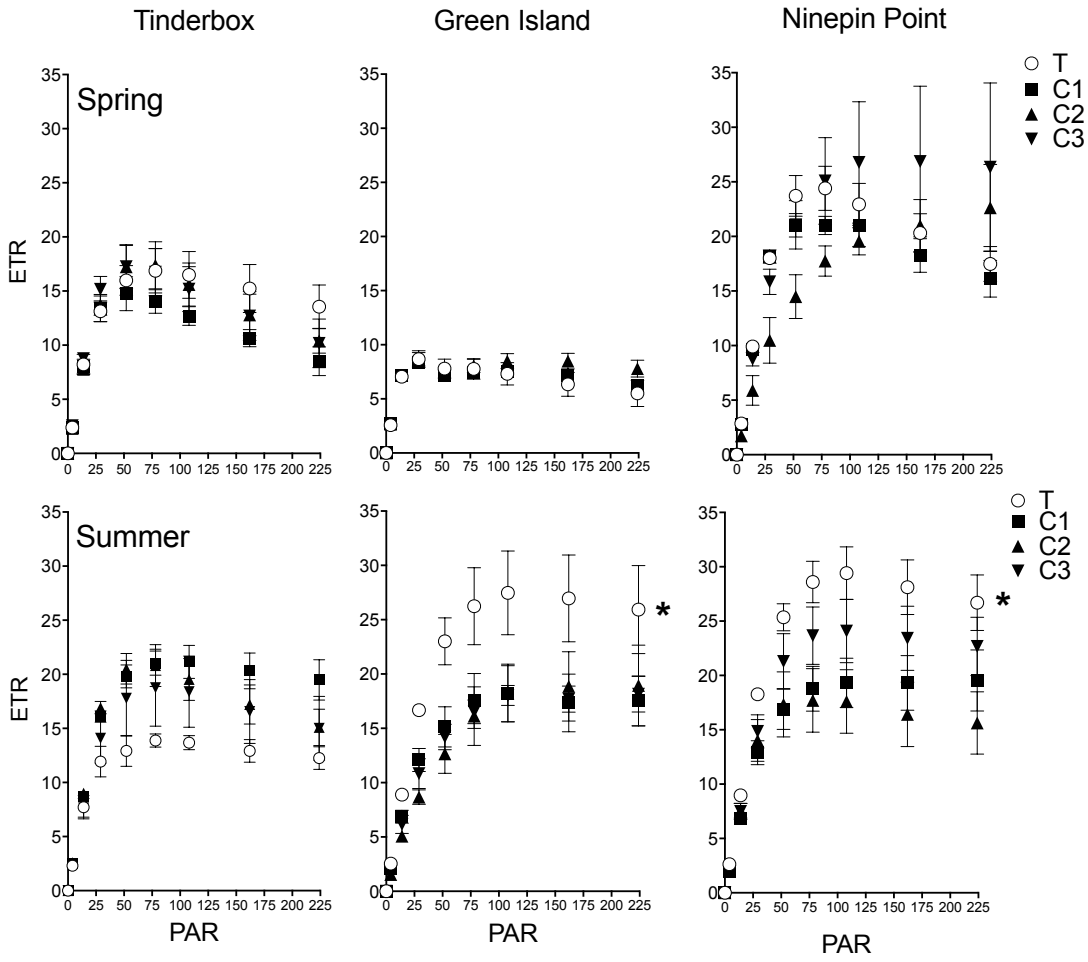

Figure 4. 9. Rapid Light Curves, (Electrons transport rate - µmol electrons m^{-2} s^{-1} - and photosynthetic active radiation, PAR - µmol photons $m^{-2}seg^{-1}$) of Sargassum fallax subjected to nutrient additions in three rocky reefs along the D'Entrecasteaux Channel. T = Treatment plots; C = control plots. The upper panel shows curves at spring (after six month of enrichment), the lower panel at summer (after nine months). Data Points are averages of five RLCs ± SE. (*) Indicates $p < 0.05$.

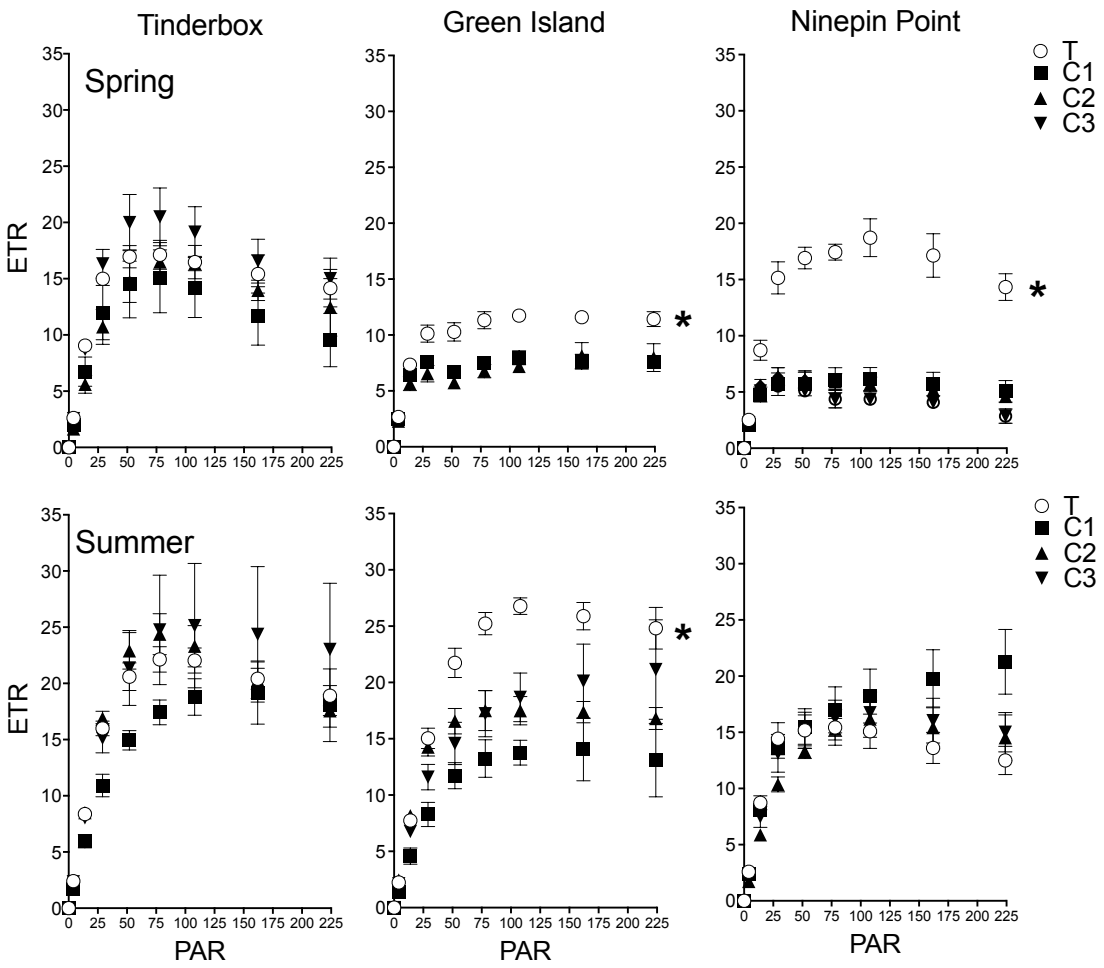

Figure 4. 10. Rapid Light Curves, (Electrons transport rate - µmol electrons m^{-2} s^{-1} - and photosynthetic active radiation, PAR - µmol photons $m^{-2}seg^{-1}$) of Ecklonia radiata subjected to nutrient additions in three rocky reefs along the D'Entrecasteaux Channel. T = Treatment plots; C = control plots. The upper panel shows curves at spring (after six month of enrichment), the lower panel at summer (after nine months). Data Points are averages of five RLCs ± SE. (*) Indicates $p < 0.05$.

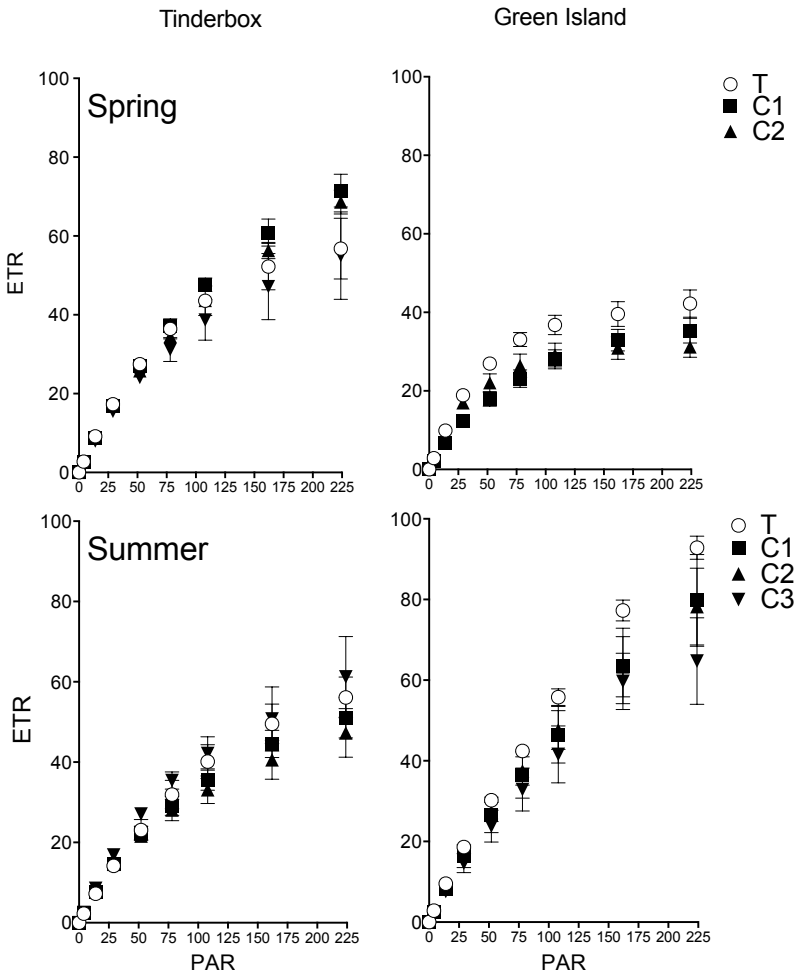

Figure 4. 11. Rapid Light Curves, (Electrons transport rate - µmol electrons m^{-2} s^{-1} - and photosynthetic active radiation, PAR - µmol photons $m^{-2}seg^{-1}$) of *Ulva* in three rocky reefs along the D'Entrecasteaux Channel. T = Treatment plots; C = control plots. The upper panel shows curves at spring (after six month of enrichment), the lower panel at summer (after nine months). Data Points are averages of five RLCs ± SE. (*) Indicates $p < 0.05$.

4.3.4. Environmental factors and physiological responses of macroalgae

Correlations between physiological responses and environmental drivers suggest that there may be some association with location specific variations of abiotic factors, and that these may vary seasonally. In general terms, the physiological responses observed in Green Island and Ninepin Pt. differed from those observed at Tinderbox in the upper Channel. This pattern of response seemed to be clearer in *Sargassum fallax* than in *Ecklonia radiata*, and was not apparent in *Ulva* sp.

Sargassum fallax.

Phosphorus was highly correlated with DIN (| r | = 0.98) during summer. In spring, the responses of Green Island and Ninepin Pt. could be clearly separated from Tinderbox by the chemical composition of *S. fallax* (Figure 4.12 A). This distinction was correlated with salinity (30% of variations) wave exposure (Openness; 5% of variations) and DIN (2.5% of variations). Light (18% of variation) appears to play a key role in distinguishing the response at Ninepin Pt. Evaluation of plant tissue composition provided some indication of treatment conditions at Tinderbox, but the observed correlation with P was weak (accounting for only 1.2% of overall variation). During summer there was no clear separation of treatment response at any location (Figure 4.12 B); Temperature explained 49% of the variation between locations, and thus may help explain the differences in physiological response (Figure 4.12 B). Wave exposure (3.5% of variations), light and DIN were good indicators of location separation, but these physical factors correlated poorly with the physiological responses (Figure 4.12 B). The photosynthetic response appears to be a better indicator of location differences in the mid/ lower

Channel in both seasons, than in the upper Channel (Figure 4.13 A and B). Wave exposure (13% of variability) and salinity (20% of variability) were the factors that best explained this separation along the first axis. The photosynthetic response for *S. fallax* suggest a treatment effect at Tinderbox and Ninepin Pt. during summer (Figure 4.13 B), and this was best discriminated by temperature and DIN, albeit these two factors only accounted for 14% of the total variability. However, light and DIN seemed to be more closely aligned with the treatment effect observed at Tinderbox in the upper Channel (Figure 4.13 B).

Ecklonia radiata.

Whilst this species showed strong location related effects on the physiological parameters, there was no clear interaction between treatment effect (as shown by *E. radiata*) and the abiotic factors measured in this study (Figure 4.12 and 4.13). The photosynthetic response of *E. radiata* also showed location specific effects. Overall, salinity was the best discriminant (31% and 35% of total variations) of observed locational and seasonal differences in the chemical composition of *E. radiata* specimens (Figure 4.12 C and D). Salinity (20% of total variability) and light (5.8 % of total variability) separated the responses at Tinderbox from the other locations during spring, whilst in summer it was the interaction between wave exposure and salinity (4.5 and 9% of total variations respectively) that best distinguished the photosynthetic response at between Ninepin Pt. from the other locations in the upper/mid Channel (Figure 4.13 D).

Ulva spp.

Ulva was only observed/ collected from Tinderbox and Green Island in this study. Whilst it seemed to be a treatment effect at Green Island suggested by the P-content (10% of total variations) during summer (Figure 4.12 F),

abiotic factors correlated very poorly with the tissue composition of *Ulva* during spring (DISTLM marginal test, $p>0.05$). Differences in nutrient tissue composition between locations were correlated with salinity in summer (40% of variations; Figure 4.13 F). In general, the separation the tissue composition of every location seemed to follow broad scale differences on abiotic factors. Photosynthetic performance between locations was a better indicator of differences in spring, driven principally by differences in light (36 % of total variability), but during summer, there was no clear separation of the physiological responses between locations and abiotic factors (Figure 4.13 F).

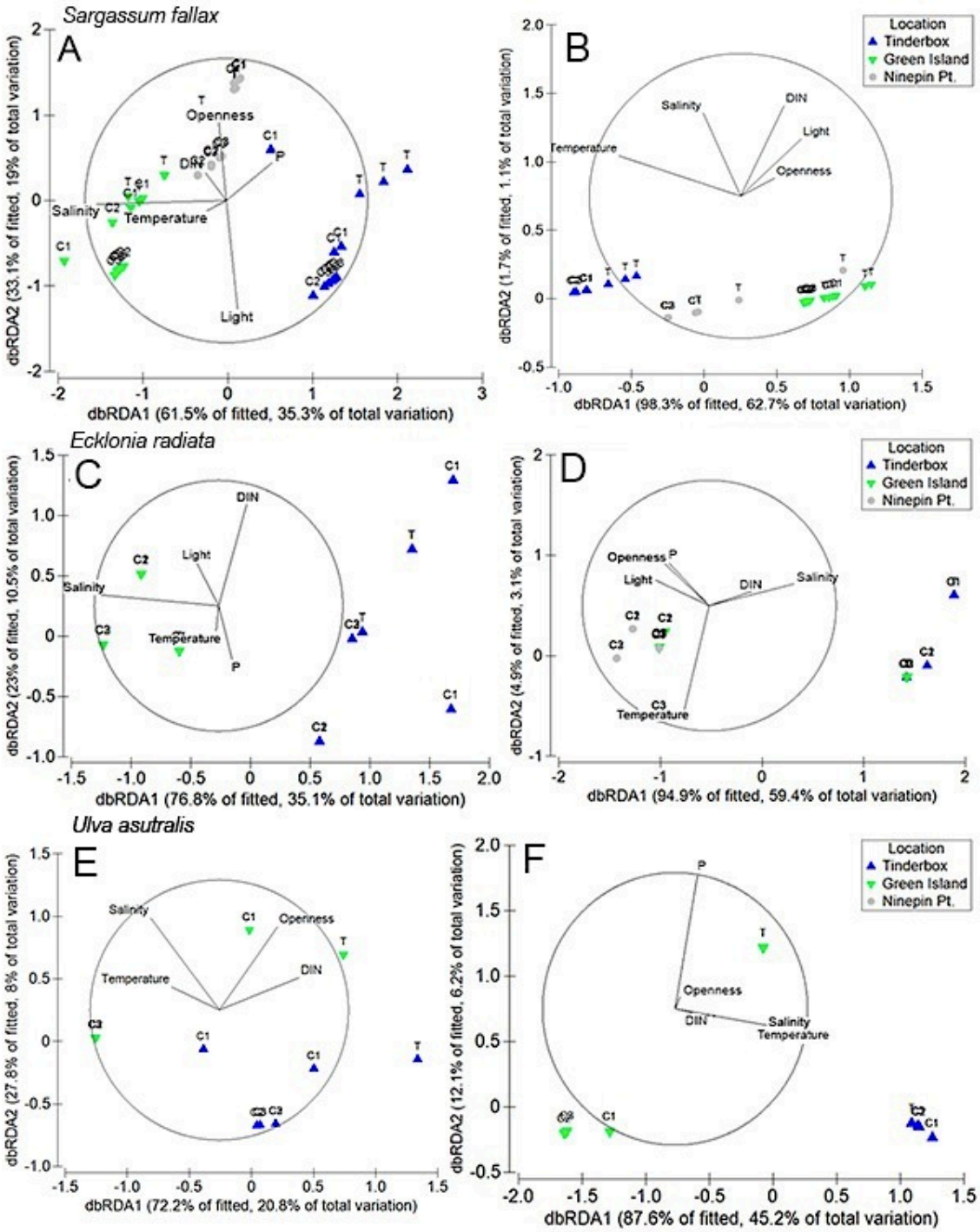

Figure 4. 12. dbRDA plot showing the responses of the chemical composition (N, P tissue content and N:P ratio) on (A – B) Sargassum fallax, (C – D) Ecklonia radiata and (E – F) *Ulva* australis during spring (left panel) and summer (right panel) in three reefs in the D'Entrecasteaux Channel. The vectors show Spearman correlations (r > 0.5) for abiotic factors best correlated with the first two axis (Circle indicates a radius of r = 1).

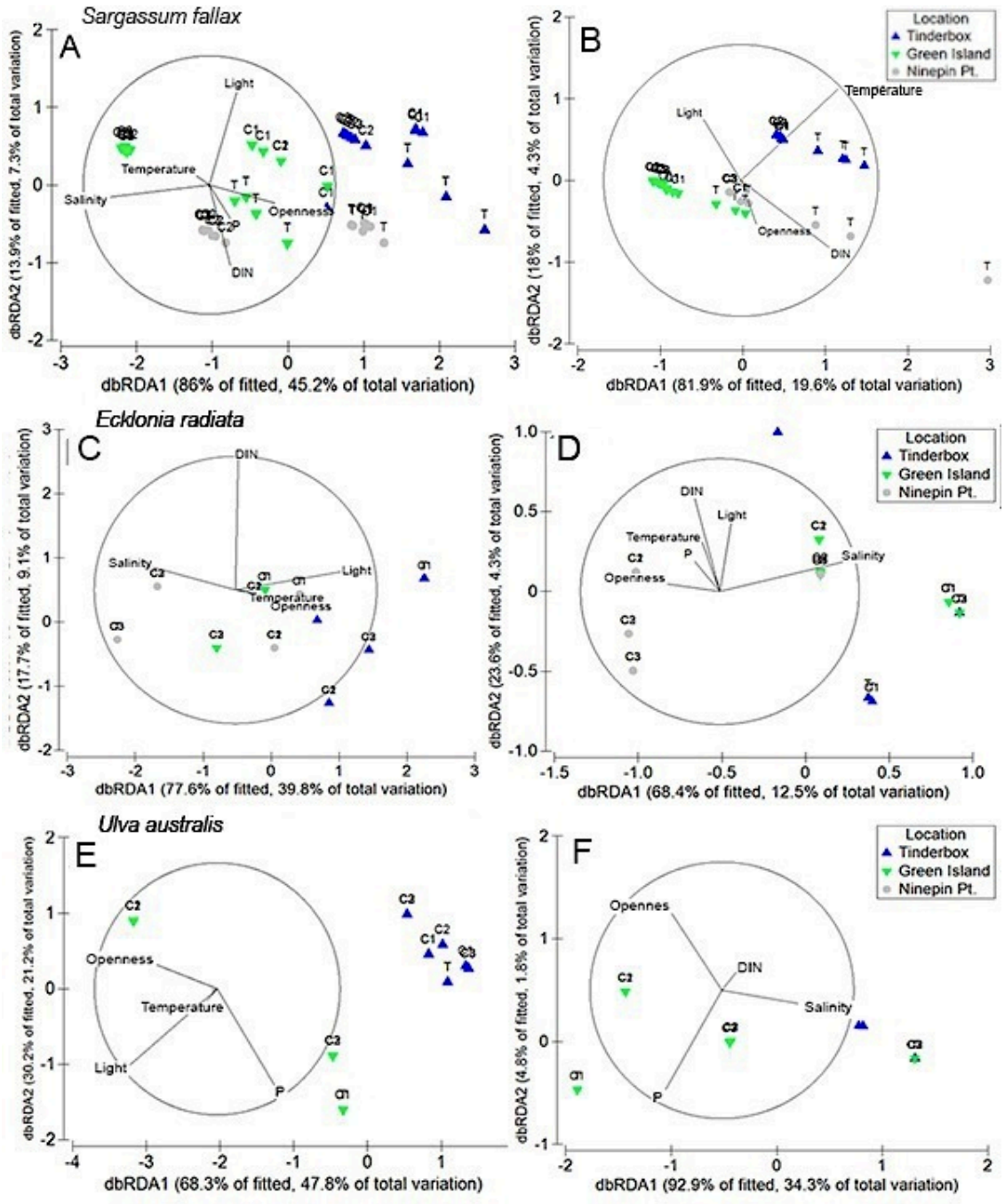

Figure 4. 13. dbRDA plot showing the responses of photosynthetic parameters (F_v/F_m, ETR_{max}, and E_k) on (A – B) Sargassum fallax, (C – D) Ecklonia radiata and (E – F) *Ulva* australis during spring (left panel) and summer (right panel), in three reefs in the D'Entrecasteaux Channel. The vectors show Spearman correlation (r > 0.5) for abiotic factors best correlated with the first two axis (Circle indicates a radius of r = 1).

4.4. Discussion

The physiological responses of the key macroalgal species assessed in this study to nutrient enhancement differed. Responses for each species to treatment conditions were context dependent, and indicated underlying location and temporally specific interactions. However, the findings do provide useful insights that can potentially be used to inform and improve coastal management.

4.4.1. Context dependency of the chemical composition of macroalgal species

The chemical composition of the macroalgae species studied here suggest that under normal conditions these species have different nutrient storage capacities that will change over time and depending on season, and that the species may also absorb nutrients differently depending on where they are located within the system. In the northern part of the channel *Sargassum fallax* and *Ecklonia radiata* had higher storage capacity (in both treatment and control specimens) than equivalent plants from the middle and lower channel. This suggests that there were sufficient nutrients available for specimens to remain close to saturation in the upper Channel. N-tissue levels from treatment locations in the upper Channel were approximately 50% higher than in control specimens from the mid/ low Channel. During summer, a time when nutrients are typically lower than spring throughout the whole Channel system (Ross & Macleod 2013), the control specimens from Tinderbox had 46-80% more N and 38-80% more P than the other two sampling locations in the middle/ lower channel, the fact that the treatment

conditions had elevated nitrogen content (more than 50%) relative to control specimens suggests nutrient limitation at these locations.

In addition, during periods of high light availability and ambient nutrient limitation, macroalgae amino acid and proteins content has been shown to decrease but carbohydrates increase (Lapointe & Duke 1984, Hurd et al. 2014). Hence, during spring/summer at temperate latitudes increases from the median C:N ratio = 18 for macroalgae (Atkinson & Smith 1983) may be expected, indicating nitrogen limitation. In addition, experimental evaluation of transient nutrient uptake rates of ammonium in *Gracilaria foliifera* and *Agardhiella subulata* suggested that the onset of nitrogen limitation could be detected when C:N ratios exceeded 10 (D'Elia & DeBoer 1978). In this study *S. fallax* subjected to treatment conditions had C:N ratios ~5 at all locations, suggesting that specimens were not nutrient limited and tissue were nutrient saturated during summer. Interestingly, control specimens from Tinderbox exhibited similar C:N ratios suggesting they were also tissue N-sufficient. A similar response was observed in *E. radiata*. This pattern of tissue saturation in the treatment and at Tinderbox contrasted with that observed in control specimens from Green island and Ninepin Pt. (C:N ratios > 20). These finding suggest that external nutrient supplies to the system more broadly at Tinderbox, presumably from anthropogenic sources (e.g., three sewage treatment plants and salmon farms), may be affecting macroalgal communities and hence uptake capacities.

These differences in the macrolagal tissue composition at each of the study locations suggest that there was a range of different nutrient regimes operating within this system. This is likely a result of the combination of differences in the supply of nutrients i.e., seasonal/episodic variations as rainfall, upwelling, and additional anthropogenic inputs from wastewater treatment plants and salmon farming. This assumption is consistent with the

recent broadscale evaluation of the ecological conditions in this region (Ross & Macleod, 2013), where differences in surface nutrient availability were attributed to the hydrodynamics of the Channel and anthropogenic inputs. Previous biogeochemical modelling for the D'Entrecasteaux Channel/ Huon estuary has suggested that ecological effects of nutrient loads from aquaculture would probably be most apparent in the upper Channel (Wild-Allen et al. 2010).

Tinderbox in the upper Channel, has three wastewater treatment plants (Howden, processing <100 kL/day^{-1} of domestic wastes; Margate, processing >100 kL/day^{-1} of domestic and industrial wastes; and Electrona, processing >100 kL/day^{-1} of domestic and industrial wastes), three finfish aquaculture sites (DIPIPWE, 2002) as well as several freshwater streams capturing a variety of different urban and agricultural catchments inputs, contributing to the background nutrient loads (Ross & Macleod 2013). These various nutrient sources would represent a relatively high "base-load" of nutrients throughout the year, and as a result it may not be surprising that specimens collected from this region might be N-replete during both seasons and consequently unaffected by treatment conditions. Experimental work on species such as *Gracilaria tikvahiae*, *Ulva rigida*, *Enteromorpha* spp. and *Macrocystis pyrifera*, have clearly shown that internal nutrient supply may change with nutrient availability (Zimmerman & Kremer 1984, Fujita & Goldman 1985, Fujita et al. 1989). In the current study the nutrient content of *S. fallax* and *E. radiata* from Tinderbox was actually higher in summer than in spring, an unexpected observation, but one which is consistent with previous studies which have suggested that storage levels may increase over spring/summer when natural sources are normally depleted and the algae usually experience high growth rates (Hanisak 1983, Wheeler & Bjornsater 1992, Lobban & Harrison 1994). These observations suggest that spatially

constrained availability of nutrients from anthropogenic sources may have promoted changes in internal nutrient pools, which in turn may result in a level of pre-adaptation in the marine flora in this area.

An alternative hypothesis may be that nutrient incorporation might respond differently to different sources of physiological stress. For instance, in limited N and P algal cultures, both limiting and non-limiting nutrient levels increased with decreasing temperature so that algal cells seemed to require more nutrient at low temperature (Rhee 1982, Duke et al. 1989). Excess of light may also promote higher nitrogen uptake to increase the xanthophyll cycle and mycosporine-like aminoacids for heat dissipation (Hanelt 1998, Figueroa et al. 2009, Korbee et al. 2010). Consequently, under low to intermediate nutrient conditions the response observed may in itself not be directly related with nutrient stress, but instead may reflect a physiological adaptation to the level of variation in the environment. However, to answer this question further research in the field is needed.

Hydrodynamic modelling for the D'Entrecasteaux Channel has shown that particles released in the upper channel (Tinderbox) tend to be retained locally, whilst those released in the Huon Estuary will be distributed homogeneously across the entire region (Herzfeld et al. 2008). In summary, the circulation patterns will tend to restrict nutrient dispersal in the upper Channel and promote nutrient dispersal in the lower channel. This may help to explain why specimens from the mid/ low area appeared unaffected by the slight increase in nutrient availability under treatment conditions during spring. If we assume that river runoff might naturally be expected to be elevated due to spring rains, then this would provide a ready supply of nutrients which would in turn keep macroalgae relatively N replete in this season. However, the response to treatment conditions was more apparent

during summer when runoffs decline (Butler 2006) and algae become naturally N-depleted, as suggested by N tissue content from *S. fallax* and *E radiata*. This suggests in turn that macroalgae from the mid/low Channel may be more sensitive to nutrient inputs in summer, and are exhibiting a response typical of oligotrophic systems, where external addition of nutrients will cause a response on primary producers (Russell et al. 2005, Russell & Connell 2007). In contrast, the upper Channel sites would appear to represent a system where nutrients are more saturated. The nutrient influences in this case comprising the combined effect of higher ambient/ local anthropogenic discharges promoted by the northern embayment circulation and enhanced by the entrained nutrient supply from the Huon River. The findings of this study suggest that the combination of physical drivers and location-specific nutrient loads have resulted in a different suite of physiological responses in this region, most notably the fact that plant tissues from this region appeared to be nutrient saturated.

The intraspecific variation observed in response to treatments conditions and the variation in extent of nutrient storage suggests that at least some perennial species may be nutrient limited at times, but that this relationship with background nutrient levels measured at any particular time may be obscured by previous nutrient storage. Overall, the gradient of nutrient acquisition observed in this system, both spatially and between species, provides a clear understanding that there are regional differences in reefs, ecology and physiology. This means that the reefs within the system need to be considered independently and that monitoring and management strategies need to take into account the prevailing environmental conditions, since the physico-biological mechanism discussed here can be attributable to the combined effect of broad-scale hydrodynamics patterns,

anthropogenic sources, and inter-specific biological attributes. This may have an impact on the productivity of perennial and opportunistic species.

Species response

The results suggest that the functional nature of the algae (i.e. rapid colonisers or late successional species) might influence their susceptibility to nutrient enhancement. Fast-growing "opportunistic" species, like *Ulva*, spp. showed greater small-scale spatial variations (i.e. patchy distribution and growth responses). Fast-growing species (e.g. sheet-like and filamentous) are well known for their rapid nutrient absorption, high growth rates and high net productivity (Littler & Littler 1980, Duarte 1995). However, these species will disappear when resources such as nutrients and light become limited and therefore are not dominant or structuring components of low/ moderate nutrient communities (Duarte 1995, Pedersen & Borum 1996, 1997). In addition, the broad tolerance of opportunistic species for small-scale changes in environmental conditions such as salinity, temperature and oxygen concentration can have a major effect on the physiological responses between sites. This in turn, may affect nutrient storage responses and may be due to factors other than changes in nutrient availability (Peckol & Rivers 1995, Fong et al. 1996, Lotze & Schramm 2000) making the interpretation of physiological changes due to nutrients difficult. Opportunists are also frequently highly affected by a range of ecological interactions such as shading, competition and grazing, or abiotic variables such as wave action, currents and light. Accordingly, the physiological performance of opportunistic species might not always be a good indicator of long-term change or susceptibility due to increases in nutrient availability where nutrient loads are moderate/ low.

Long-lived macroalgae (e.g., *S. fallax* and *E. radiata*) are better adapted to grow under low nutrient conditions, taking up nutrients efficiently when they become available and storing them for future use (Chapman & Craigie 1977, Fujita 1985, Duarte 1995, Pedersen & Borum 1997, Pfister & Van Alstyne 2003). Consequently, perennial macroalgae will reflect the integrated impact of past nutrient regimes (Fujita 1985, Lapointe 1985, Wheeler & Bjornsater 1992). Some have argued that this may obscure short-term changes (peaks) in external nutrient sources (Kim et al. 2014), but whether this is an issue will depend on the question being asked i.e. whether the aim is to assess short- or long-term structural viability in highly altered or more pristine reef systems. In addition, long-lived species can reflect differences in nutrient regimes at low nutrient concentrations in a broader temporal scale (Pedersen & Borum 1997, Thornber et al. 2008). Their permanence in the system gives slow growing algae the competitive advantage in areas with fluctuating nutrient regimes (Pedersen & Borum 1996, 1997). Understanding changes in the background nutrient status (and related algal species) may be especially relevant in systems where response to the eutrophication process may be confounded with natural variability (Krause-Jensen et al. 2008). In the current study the internal nutrient content of *S. fallax* seems to reflect the nutrient variability within this system and responded to location-specific variations. This suggests that *S. fallax* would potentially be a good indicator of long-term condition status in the D'Entrecasteaux Channel.

4.4.2. N:P ratios in the Water column

Examining the N and P composition of the water column can provide an understanding of not only the nutrient status but also of any potential limitations on algal growth (Lapointe & Clark 1992, Wheeler & Bjornsater 1992). The maximum seawater N:P ratio in the current study was 9, which is

considerably lower than the Redfield ratio (N:P = 16) which defines normal expectations for marine waters (Redfield 1958, Atkinson & Smith 1983, Wheeler & Bjornsater 1992). Consequently the water column would seem to be N-limited during spring/ summer. This is consistent with previous observations in this region (Ross & Macleod 2013).

Variations between locations and over time seemed to reflect the established hydrographical differences within this system (i.e., the circulation limitations in the upper Channel and the higher level of mixing and seasonal influences in the mid/low Channel). Even though deviations from the Redfield ratio may be expected in estuarine systems as a result of natural changes in freshwater inputs (Atkinson & Smith 1983, Wheeler & Bjornsater 1992), anthropogenic discharges will tend to provide an additional signal (Lapointe et al. 1992). For example, intensive salmon farming has been shown to enhance the availability of nitrogen in the water column increasing N:P ratios in the proximity of salmon cages (Folke et al. 1994, Troell et al. 1999), whilst agricultural runoff, rich in P, may decrease the N:P ratios (Paerl 2009). The results of the current study indicated that conditions in the mid/ low Channel tended to be nutrient limited, with water column ratios generally "un-saturated", and as a result this region might be expected to be more sensitive to "external" nutrient additions. Additionally, there was a seasonal component to the water column observations at Ninepin Pt. (the low Channel area) that could be significant.

4.4.3. Photosynthetic activity

In this study we found that the descriptive parameters of photosynthesis (ETR_{max}, F_v/F_m, E_k) provided no additional insights on the potential effects of nutrient additions on plants from treatment sites. Given that a significant

increase in water column concentrations were not detected beyond 1 m from the sources (See chapter 2 for further discussion), the responses may have been somewhat obscured by the enrichment regime, and small-scale spatial variability between samples. Previous studies on a diverse range of macroalgae (e.g., *Ulva lactuca*, *U. rigida*, *Gracilaria chilensis*, *Macrocystis pyrifera*, *Sargassum stenophyllum*) have shown that nutrient enhancement usually produces an increase in photosynthetic activity either through O_2 evolution (gross photosynthesis) or via ETR_{max} (Longstaff et al. 2002, Cabello-Pasini & Figueroa 2005, Abreu et al. 2009, Figueroa et al. 2009). Nutrient availability has to exceed certain threshold values to effectively increase the total amount of RuBisCO (and hence photosynthesis), especially in nutrient limited environments (Bird et al. 1982, Horrocks et al. 1995, Pedersen et al. 2010, Lobban & Harrison 1997, Wykoff et al. 1998). Nevertheless, the trends observed in the RLCs imply potential physiological differences since they were for the most part associated with equivalent rises in N-tissue content. The broad-scale differences observed in the chemical composition of macroalgae were reflected in differences observed in the RLCs of specimens from mid/low Channel during summer. In this example the photosynthetic response of *S. fallax* was found to have elevated N storage (N:P ratios > 40) and high C:N ratios (i.e., suggesting N-sufficient tissue). Overall, these results suggest that the rapid assessment of macroalgal photosynthetic activity in situ may be a useful complementary tool for monitoring. However, the interpretation of these responses may require further investigation since some studies have criticised the effectiveness of this method in algae under nutrient limiting conditions (Kolber et al. 1988, Falkowski & Raven 2007).

4.4.4. Interaction of physiological responses with abiotic factors

As already discussed, the hydrodynamics of the D'Entrecasteaux Channel/ Huon Estuary provide a broad-scale mechanism for nutrient dispersal that

may influence nutrient residence time and diffusion. It is well established that hydrodynamics can have a significant influence on the physiology of macroalgae (Hurd 2000). In addition, light, water motion, nutrient availability, temperature and salinity can all also have a critical influence on the extent and rate of physiological processes in macroalgae (Hurd et al. 2014). Although the results of tissue composition in the current study identified a treatment response during summer (Figure 3 A, 4 B), there was little evidence of any particular correlation with environmental variables. DIN, light and wave exposure appeared to have a level of location-specific treatment interaction (Figure 13 B), but again their association with environmental variables was unclear. Similarly, although the photosynthetic performance (RLCs) suggested a treatment effect during summer, correlations with environmental drivers (i.e. temperature, DIN, light and wave exposure) were unclear, possibly as a result of low level of spatial resolution in the measurement of these parameters (Figure 14 B). The relationship between nutrient storage capacity and location may in fact be quite a complex interaction involving not only the inherent nutrient pool but also variation in a range of local physical processes. Consequently, future research should focus on examining the influence of these different environmental drivers and how they may change spatially within the system.

4.4.5. Summary and recommendations for further work

This study intended to look at the effects of a nutrient source against a more generalised and spatially varying background of nutrient inputs simulating the monitoring of effects of a localised but disparate nutrient source such as a salmon farm or Sewage Treatment Plant on a reef system. Although the measurement of specific nutrient outputs, perhaps at source, it seems an alternative way to characterise the potential impact, this may not necessarily

take into account differences in the background nutrient load, and so may result in confusion with the interpretation of effect. In this case we chose to measure the response of macroalgae in association with background load to provide a more representative understanding of “real world” conditions.

Tissue nutrient content has previously been proposed as a suitable indicator for detecting the effects of sewage and shrimp aquaculture effluents on the water quality in tropical waters (Fong et al. 1994, Costanzo et al. 2001, Fong et al. 2001, Jones et al. 2001, Costanzo et al. 2004, Lin & Fong 2008, Kim et al. 2014) but there is less evidence of this for temperate systems and where studies exist they tend to be linked to highly altered conditions (Lyngby & Mortensen , Lyngby 1990). To our knowledge, this is the first study, which has used physiological responses as indicator of the status of the system and effects of moderate nutrient loads on temperate rocky reef systems at a realistic scale.

The physiological response of the reef in the upper channel suggests that the plants in this region were subject to a higher background nutrient loading, and that some species were “nutrient saturated” compared with those from the mid and lower Channel. As a result the reefs in the mid/lower channel might provide more sensitive indicators to increasing nutrients levels in the system as a whole, whilst the C, N content and C:N ratio of plants in the middle region might provided a better indication of how close the system might be to saturation, and thus potential tipping points for change in the broader communities. Further research is needed to establish just where the tipping point (Scheffer et al. 2001) might be for these communities and how other factors (e.g. physical and ecological interactions) might contribute to the potential for a shift to an alternative ecosystem condition. These findings suggest that the inclusion of physiological indicators could improve the sensitivity of coastal reef monitoring. Being able to include a more accurate

measure of macroalgal response (including the spatial and temporal variability) in coastal ecosystem models could provide a better understanding of the system nutrient status and allow managers to better predict potential impacts on macroalgal communities.

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5. Chapter 5. General Discussion

5.1. Effects of nutrient enrichment on macroalgal-dominated habitats

In this study, N and P concentrations were experimentally manipulated deploying 400kg of slow release fertiliser directly on reef parcels of 100 m^2. Elevated nutrient concentrations (10-fold ambient levels) were detected ~10 cm from the supply units similarly to previous field experiments in marine fertilization at smaller scales (See Hatcher & Larkum 1983, Lapointe 1989, Coleman & Burkholder 1995, McGlathery 1995, Miller & Hay 1996, Worm et al. 2000, Russell & Connell 2005, Russell et al. 2005, Russell & Connell 2007, Teichberg et al. 2008, Teichberg et al. 2010). There was little community change observed over the study period, suggesting that the community was relatively resilient, was not nutrient limited or that the enrichment did not work over such time scales (i.e., the communities may or may not be nutrient limited). However, when we consider not just the community structure but also the physiology and prevailing environmental conditions, the "weight of evidence" suggests there may be differences between the locations that could be used to infer spatial patterns in sensitivity/ resistance. There were differences in the physiology of key species that indicated they were being affected by nutrient additions. The results suggested that perennial species were able to store nutrients during winter peaks (or from random flood or surge/upwelling events) as a result might not respond to N stimulation during summer nutrient depletion if they were already saturated. However, this effect differed between locations, which in turn, implies that environmental conditions can influence response. These findings suggests that it is critical to consider the specific environmental conditions at each location as this will determine the environmental sensitivity or resistance of each reef system.

This study in turn suggests that monitoring and management strategies need to identify where ecosystem change is likely to occur and what location-specific drivers for this change may be.

Reefs communities are able to compensate for certain level of nutrient variability, and therefore, unless the nutrient inputs exceed a threshold level any change in ecosystem function is likely to be relatively minor and the response will be slow (Bokn et al. 2002, Bulleri et al. 2012). Most monitoring programs focus on detecting major changes, which means the system has already been altered. To our knowledge, there seems to be scarce documentation of potential indicators of longer-term systemic change; these indicators are needed if we want to influence management of likely system responses before major change occurs.

5.2. Opportunists as the first indicator of nutrient enrichment

Opportunistic and fast-growing macroalgae are frequently used as indicators of nutrient enrichment (Schramm 1999, Juanes et al. 2008). There are well-established relationships between opportunist proliferation and environmental deterioration (Gorgula & Connell 2004, Russell et al. 2005, Salas 2006). Whilst opportunists may appear rapidly in response to increases in nutrient availability, they often disappear just as quickly. It is when they remain long enough to inhibit other species or when they completely dominate the community that changes in ecosystem function will be observed. For instance, experimental evidence have shown that the change that precedes seagrass loss due to opportunistic epiphytic flora occurred late in the process of eutrophication and usually too close to the ecosystem

turning point to provide an early warning (Schramm 1999, Cambridge et al. 2007).

In this study, the opportunistic species presented considerably small-scale temporal and spatial variability. They appeared to be more frequent and abundant at mid Channel sites, but as a result of the small-scale patchiness this relationship was not statistically significant. Since this may reflect a broad-scale effect of abiotic conditions on communities, opportunists may actually play an important role in "mopping up" excess nutrients. Some studies have suggested that opportunists can be key drivers of ecosystem change, but the rate of response will be influenced by the prevailing conditions at each individual location and ultimately, the natural carrying capacity for that location will determine the impact. Therefore, exploring and understanding the natural mechanisms influencing opportunists at a given location will help identify potential gradients of resistance/resilience and clarify those locations where the release of nutrients may represent a higher risk for macroalgal reefs. Opportunists, although an important short-term indicator of nutrient enrichment, do not necessarily represent a good long-term indicator of changes in environmental function. For this reason they need to be viewed in the broader context of the spatial and temporal environmental conditions that determine the local community structure.

Opportunistic, fast-growing species are common annual components of coastal reefs communities (Littler & Littler 1980). They indicate increases of nutrient availability, but incidentally their occurrence also implies a process of incorporation of available nutrients. In this sense, they become part of the ecosystem "filter" (Sensu Cloern 2001). Although their presence may suggest an increase in nutrient concentrations natural variability may make impossible to translate this to a direct estimate of susceptibility of reef

communities, unless substantial abundances are reached. At the broader ecosystem scale the spatial and seasonal variability may prevent opportunistic species acting as an early indicator of nutrient enrichment. We need to be careful that in using opportunists as an indicator we ensure that any response associated with that indicator capitalises on the opportunist's nutrient assimilation capacity and works in the context of the carrying capacity of the system.

5.3. The influence of environmental conditions can mask effects of nutrient enrichment on macroalgal reefs

Wave action, the light environment, salinity, temperature and nutrients have the capacity to affect the structure of macroalgal communities but can also influence the community's susceptibility to nutrient enrichment (Eriksson & Bergstrom 2005, Kraufvelin et al. 2006, Kraufvelin 2007, Krause-Jensen et al. 2007a, Wernberg & Connell 2008). Light level is a critical determinant of macroalgal reefs communities and will fundamentally influence the balance of green, red and brown algal species. Light is strongly autocorrelated with depth and together these factors will determine changes and patterns in vertical distribution. Freshwater inflows, sediment resuspension, wind, local currents and wave action can also influence light attenuation (Hurd 2000, Denny 2006). Light levels are frequently used to indicate water quality, with light attenuation often associated with turbidity (Krause-Jensen et al. 2007a, Krause-Jensen et al. 2007b), also it has been shown on many occasions to be strongly aligned with the macroalgae responses to nutrient enrichment (Valiela et al. 2000, Tomasko et al. 2005, Krause-Jensen et al. 2008). Given that light is such a key driver of macroalgal communities it would seem reasonable that it might be included in any suite of local characterization

measures. Similarly, salinity and temperature also have shown to be linked with nutrient enrichment at geographical scales (Krause-Jensen et al. 2007b) and can influence the individual performance of opportunistic species at finer scales (Fong et al. 1996, Kamer & Fong 2000). However, the relationships between nutrient enrichment, salinity, temperature and impacts on macroalgal assemblages will vary (Krause-Jensen et al. 2008), as these parameters may also vary within estuarine systems as a result of fresh water inputs (Atkinson & Smith 1983, Wheeler & Bjornsater 1992). In addition, other factors such as wave exposure may increase dislodgement and export of opportunistic algae in shallow reefs (Krause-Jensen et al. 2007b). Consequently, interpretation of macroalgal community change based on opportunistic responses may be limited by specific abiotic effects inherent to the system (Painting et al. 2007, Krause-Jensen et al. 2007a, Krause-Jensen et al. 2008). In this study, these association of environmental variables (e.g. light, wave exposure, temperature, salinity and nutrient loads) and the differences observed in the community composition during the experiment's duration suggested that once again, it is clear that prevailing environmental conditions (location) play a key role in determining (1) what the community looks like (2) how the communities might respond to impacts, and that (3) this will influence to what extent we can predict that response.

5.4. Biophysical combined effects.

Light, salinity, temperature, nutrient regimes although individually important drivers of community structure do not act independently, they work together to make up the environmental conditions at a location at any given point in time. Consequently, if it was possible to define a single factor that adequately represented this combined effect this could be used as a proxy of "environmental conditions". From the results of this study wave exposure may

be a potential candidate. This study showed a clear gradient between the community structures at each location that could be related to exposure, and therefore used to characterize the underlying location specific environmental conditions. Large-scale geographical variations in wave exposure have shown to be correlated with macroalgal community structure (Burrows et al. 2008, Hill et al. 2010). Certain taxa characterize sheltered environments such as Caulerpales, whilst others such as *Phyllosphora comosa* and the encrusting algae *Lithothamnion* sp. are more indicative of higher wave action environments (Edgar 1983a, Sanderson & Thomas 1987, Denny 2006). Since this was very much shown to be the case in this study, the effect of wave exposure combined with competition for resources such as nutrients and light, highly influenced by canopy-forming species (Duggins et al. 1990), stresses the importance of wave exposure shaping the community composition. In turn, community characteristics may act as a modulating factor for benthic primary production (Eriksson et al. 2006b) and consequently on the potential impact of ephemeral species of macroalgae (Eriksson et al. 2007).

Whilst this study did not show a clear relationship between exposure and nutrient enrichment effect it was clear that the underlying community at each location could be defined according to the level of exposure and that those communities differed in their response to nutrient enrichment. Consequently, exposure may be considered a useful tool to capture the locational differences that might predispose or sensitize a community to nutrient enrichment. Given that differences in wave exposure will result in different levels of physical stress on macroalgae (Denny 2006, Wernberg & Connell 2008), some communities may be more susceptible to the additional stress of nutrient inputs since the degree of exposure might influence the tolerance levels. Thus, less exposed sites might be subject to less variability in

environmental conditions than more exposed sites and thereby increasing levels of susceptibility.

Patchiness in reefs (high levels of local scale spatial variability) has been shown to be indicative of habitat disturbance (Caswell & Cohen 1991, Warwick & Clarke 1993, Terlizzi et al. 2002). The current study observed a gradient of patchiness in the reefs that appears to be driven by changes in the underlying community structure, and particularly changes in opportunists that might reflect a stress response. However, this may also be affected by the prevailing environmental conditions and hence further research is needed to establish i) a clear causal effect and ii) whether this observation is temporally consistent and therefore could be used as an index of risk and vulnerability.

One other factor that needs to be considered is grazing; whilst not directly assessed in this study, previous research has shown that grazing can control proliferation of fast-growing species (Hauxwell et al. 1998, Russell & Connell 2005) and may increase resilience in reef ecosystems (Scheffer et al. 2001). In Tasmania, the abundance and diversity of grazers has been directly associated with the prevalence of understorey/turf-forming species (sensu Edgar et al., 2004), and as an important habitat modifying specie of canopy algae distribution (Ling 2008, Wernberg & Connell 2008, Connell 2008). Thus the combined relationship between multiple regional differences in ecological patterns (e.g. herbivory) and physical factors as wave action can create processes that may indirectly provide a further source of resilience/resistance in macroalgal reefs within the system that needs to be considered in monitoring designs.

5.5. Context dependency

As discussed previously the results indicate that greater levels of wave exposure and light attenuation seem to result in lower nutrient impacts on reefs communities. Whether this is as a result of greater dispersion of nutrients, physical abrasion/dislodgement of ephemeral algae in more swell exposed environments and lower light levels reducing the overall impact, or a more complex interaction resulting in a community that is more resilient is hard to categorically establish. However, perhaps the mechanism doesn't matter if we can still establish a monitoring/ management strategy that inherently account for these factors. What the findings do suggest is that a different scenario for management and monitoring may be needed in the lower Channel as compared to the upper Channel based on prevailing abiotic attributes and fundamental community differences underpinned by such environmental features.

The reefs in the upper Channel are already subject to a higher level of anthropogenic impact than the other reefs in this study (Wild-Allen et al. 2010, Ross & Macleod 2013). The results suggest that whilst they did not respond with "classical" indicators of enrichment (i.e. opportunistic species), there was evidence of stress at a physiological level in key structuring species. It may be that the underlying stressors in the upper Channel have already determined the reef community structure in this region. Opportunists were present at all sites, supporting the assertion that the Channel system as a whole was subject to similar level of nutrient stress. Consequently, opportunists may be poor indicators of further change in this system, and more reliable measures will be needed to take into account the fact that the system may already be stressed. This may have to involve subtle measures such as physiological response. However, this is assuming that our end point

is to measure change in the system from a current base point and not to assess the total impact, as the data suggest the system is already stressed. Evaluation of the overall condition of the system as stands may be more informative for management and assessment of carrying capacity, because it may reflect longer-term differences of biological responses to existent nutrient conditions.

In contrast, the reefs of the lower Channel were subject to more dynamic environmental conditions and hence greater dispersion or diffusion of any nutrient impacts. There was also a more pronounced seasonal effect at this location. As with the upper channel it was very hard to distinguish a clear year-round indicator of nutrient effects, but I would propose that the mechanisms in this case are quite different; the lower channel communities appear to show little or no predisposition to nutrient inputs, and the environmental conditions were such that any nutrient signal had to be strong in order to be detected. That said the findings did show a clear summer increase in opportunists, which is what might be expected naturally; this emphasises the point that understanding the natural (present) response patterns is important in order to accurately assess the significance of any deviations from that pattern. This also highlights that a different monitoring/ management approach may be needed in the upper and lower Channel, and once again we could ask the question, “Does the carrying capacity differ”?

5.6. Physiological responses as indicators of change in nutrient availability

Although tissue levels of nitrogen in macroalgae from the upper Channel were consistently higher than other locations, they did not respond to the additional nutrient load. If we accept that the upper Channel is already impacted then we may be looking for an impact signal on top of an existing

impact, which will be harder to establish, and the levels in this area suggest that the plants are already nutrient saturated. Perennial species as Sargassum fallax have the capacity to store “luxury” nutrients when available (Fujita 1985, Pedersen & Borum 1996) and this was evident in the upper Channel, but more importantly, this saturation level was apparent in summer when it might be expected that the system would be nutrient limited. On the other hand, the mid/lower Channel sites did respond to nutrient additions in summer as expected (Plants were unsaturated, so they incorporated nutrients only in the treatment plots). Consequently, understanding not just the natural environmental conditions but also the anthropogenic influences in a given location is important in establishing a monitoring or management program and reinforces the previous observation that “one size does not fit all”. It may be necessary to refine monitoring approaches for areas with existing impacts to be able to detect more subtle changes and in this case physiological responses may be more appropriate. Classical indicators can give an earlier warning of a change in the system but are only really useful if you can contextualise their response against their natural variability. In areas where impacts are already present monitoring will necessarily be focused on managing any further deterioration and avoiding tipping points

5.7. System vulnerability and monitoring: What type of monitoring will be suitable?

In this study similar dynamics evident in both treatment and control plots at each location evidenced no significant impact of experimental nutrient addition on the community structure in the mid/lower Channel. However, there were physiological responses at treatment conditions that suggest a reaction to nutrient enhancements. These systems had higher wave

exposure, natural cycles in light attenuation processess and a nutrient regime that was driven by natural hydrodynamics that was consistent with normal “oligotrophic” conditions. This system appears to be more resilient to nutrient enrichment primarily as a result of this hydrodynamic regime. In contrast, the upper Channel reefs systems showed symptoms of nutrient stress. Whilst these reefs still functioned effectively and the communities appeared normal, there were no physiological differences to nutrient enhancements. However, nutrient levels in the algae were much higher than found in the lower Channel. This may be indicative of background long-term nutrient levels. Accordingly, this area may be subjected to longer-term successional process of change caused by nutrient enrichment and thus present a higher risk of, for example, a abrupt rise in abundance of opportunistic species. This also once again suggest that different management approaches may be appropriate in this region, and that this management approach should take account not only the existing environmental conditions but should also consider future risks scenarios. In this last section I propose a strategy for monitoring/ management of the upper and lower Channel independently (Figure 5.1).

5.7.1. Monitoring Scenario 1: Tinderbox, upper Channel

At this location the results showed that physiological responses were the result of and environment subjected to more nutrient availability. Therefore, it is important to accurately monitor physiological variations. Assessment of the nutrient content of perennial species as Fucoids may be particularly useful for defining longer-term nutrient regimes since these species allow the monitoring of nutrient content in a round year base. In addition, monitoring of nutrient levels in opportunistic species may help to clarify critical seasonal

variations (e.g., seasonal nutrient depletion and increases in light - summer). Multiple samples from a greater range of sites across the system, and at finer temporal resolution will be necessary in order to better define those locations most at risk ("hot-spots") and providing a geographical context for potential differences/ impacts as well as any temporal sensitivity. The current study was only able to characterise a single reef in this region, but the results suggested that this reef was already nutrient impacted, consequently it will be important to determine if the other reefs in this region are at the same level or if there are areas of higher/lower impact. In addition, it will be important to identify the prevailing physical drivers (i.e., water transport, wave action, light attenuation) as these may increase or constrain the effect of the current nutrient loads and will provide important management context. I would propose monitoring of water nutrient levels in conjunction with the algal sampling, but also in areas with known anthropogenic inputs, in order to better characterise the spatial risk profile. As we have already established that the community in this area is stressed I would also propose a level of community monitoring is included, to provide early warning of any potential point (e.g., tissue saturation in periods of natural nutrient depletion, major changes in percentage of cover of canopy-forming species, filamentous and/ or ephyphitic algae loads, increased habitat patchiness). Whilst not part of monitoring per se it would be valuable to undertake additional research on the effect of grazing and any potential synergistic interactions with nutrient enrichment as this has been reported to increase community resilience. Similarly, sedimentation can have a major effect on reefs through a whole variety of interactions and so should be considered/ monitored in much the same way as the physical drivers. Given that this system is already stressed perhaps future management should be looking at how to reduce nutrient inputs in the upper Channel. Whilst this strategy may be costly the risk to the system of not doing it may be considerably more expensive.

5.7.2. Monitoring Scenario 2: Green Island/Ninepin Pt., mid/lower Channel

The results from the mid/ lower Channel sites suggested that this region was less stressed than the upper Channel, but that increases in ambient nutrients (stress) could be readily detected by monitoring plant tissue nutrient load. Whilst there was no major change on community structure at either of the study sites and we did not detect any additional stress with nutrient additions, it would be unrealistic to assume that these communities are not affected by nutrient enrichment. There were physiological differences between sites, both with nutrient addition and seasonally, which suggest that there were subtle but real changes in the system were subtle but real. The findings also suggest that the community from the mid Channel was perhaps more sensitive than that of the lower channel and represented an intermediate stage, in both community structure and environmental conditions (i.e. wave exposure). However, it is important to acknowledge that we only assessed two locations in this region and as with scenario 1, I would recommend that further sites be monitored to better characterise the spatial variability and confirm the findings outlined above. Assuming that the patterns described are real it might then be reasonable to have a reduced level of monitoring in this region. In fact if the gradient hypothesis is correct then the mid Channel location may by itself provide the most reliable indication of changes in the system as a whole, and could act as a “canary in the coal mine”. However, before taking this approach we would need to be confident that the assumptions are correct and that we would not miss a tipping point elsewhere in the system.

5.7.3. What can these findings tell us about “real world” management issues?

Firstly, the findings of the surveys of established and the successional communities showed no evidence of major changes as a result of nutrient inputs and the species mix was consistent with what we would normally expect to see in these areas. The community structures even suggested that the systems were relatively stable. However, there were responses at a physiological level that could prove useful as an early warning indicator for management, to help identify sensitive areas within the system and could perhaps provide guidance on carrying capacity. A key objective of this research was to provide a better understanding of the interaction between anthropogenic nutrients and reef health. One of the key nutrient sources in this system is finfish aquaculture. Salmon farming is well recognised as being a significant source of readily available nutrients in the water column and its effluents will mix with other anthropogenic sources in the receiving environment (Troell et al. 2003), which may increase the overall impact (Troell et al. 1999). Being able to provide reliable early indications of potential adverse interactions in a time frame that allows a management response is essential for this economically important industry to be sustainable.

There is a general perception that salmon farms should not be located “close” to subtidal reefs systems, but a key question is how close is too close? But more specifically in the context of this study the question could be “where” close is too close? Elevated concentrations of ammonia have been detected in the water column 200-300 m from salmon farms in studies from Norway (Kutti et al. 2007) and Scotland (Sanderson et al. 2008), whilst particulate organic waste in Norwegian fjord systems have been detected up to 500-900 m (Kutti et al. 2007). However, direct impacts on nearby reef systems seem to be difficult to determine. The closest salmon farm to the

reefs in the current study was 900 m (Tinderbox, upper Channel). At the current nutrient levels observed in this study there was no change in the community structure that might suggest the function of the reef was at risk, so that may lead to the assumption that a kilometre is a reasonable distance. The results of this study clearly showed that the biophysical conditions at all of the reefs studied caused a pronounced dilution in nutrient concentration from 10 cm to 1 m, which may suggest that any observed effects were not solely a function of proximity to nutrient source, but of background conditions, both in terms of physical influence and other impact levels as tissue nutrient content. For example, salmon farms in the D'Entrecasteaux Channel/ Huon Estuary are located as close as 150 m to coastal/ fringing reef but potential impact at each reef should be considered according to its individual environmental risks characteristics, which could modify the critical distance at which nutrient effects may be detected.

One of the other key concerns often expressed is what is the carrying capacity of the system? This study did not specifically address this question, however, the findings are relevant in that i) whilst there was indication of low level stress in all three reefs communities, the system appears to be coping (i.e. within carrying capacity) ii) The upper Channel showed evidence of greater sensitivity than the lower Channel, that may be attributed to higher levels of cumulative impacts in this region (i.e. spatial differences in carrying capacity) and iii) location may influence sensitivity/ resilience (i.e. spatial differences in carrying capacity).

Using the calculations outlined in Mente et al. (2006) the nutrients added in this study can be considered as equivalent to the nitrogen waste outputs associated with the production of approximately 900kg of salmon. This would effectively increase the ambient nutrient levels by 10 times, based on the mean values of 1.5 – 13 μM of total nitrogen measured in the vicinity of the

nutrient sources (Figure 5.1). These concentrations are comparable to nitrogen levels reported from next to salmon farms (0 - 4 m) in Canada, which ranged between 8.6 – 9.5 µM (Wildish et al. 1993), and in southern Chile, where nitrogen concentrations in the vicinity of salmonids farms (~ 100 m) reach ca. 30 µM (Buschmann et al. 2008). Interestingly, this level of nutrient enhancement caused negligible effects in the macroalgal community structure included in this study. Salmon farming in the D'Entrecasteaux Channel and Huon Estuary presently produces ca. 27,000 tonnes of fish per annum (pers. comm., TSGA 2015), which equates to approximately 3,500 tonnes of N dissolved waste outputs (Islam 2005, Wang et al. 2012). Clearly, there is a high level of natural dilution and diffusion of these nutrients in this system, as shown by the rapid depletion of the nutrients in the current study. Given the lack of change detected in community structure following nutrient additions it would seem that the reef ecosystems in the D'Entrecasteaux Channel and Huon Estuary might exhibit certain levels of resilience. However, the differences in physiological response in upper and the lower Channel would tend to suggest that this resilience can be tempered by the prevailing environmental conditions, and that these additional nutrients may be influencing the overall carrying capacity of the system. This could in turn imply that areas with pre-existing or higher nutrient loads (e.g. the upper Channel) may have a reduced carrying capacity. This reinforces the need to understand the prevailing conditions at each reef (i.e. the natural and anthropogenic inputs and dynamics) to ensure that monitoring and management actions are risk appropriate and may also suggest that these systems might need to differ in the types of management/ monitoring employed (See Figure 5.2).

Many studies have shown that an important fraction of these nutrients is incorporated by phytoplankton (Anderson et al. 2002) and consequently

phytoplankton/ chlorophyll levels are often used as indicators of salmon farm impacts. However, not all studies have detected increases in Chlorophyll α in the vicinity of salmon cages (Pitta et al. 2009), which suggest that chlorophyll may not always be a reliable monitoring tool. One other concern with using phytoplankton as a monitoring tool is that phytoplankton communities are transient, would be influenced by background environmental conditions and actually respond to episodic increases in nutrients rather than cumulative/ integrated effects; reef monitoring can provide that cumulative understanding.

The current study suggested that opportunistic species, the classic indicators of nutrient enrichment, were not very reliable and provided little understanding of subtle changes in the system as a whole. These indicators are much more effective for major impacts and the findings suggest they should always be viewed in the context of the local environmental conditions. Consequently, what can we use to give warning of system wide changes in a time frame that will allow realistic management action? This study suggests that monitoring of changes in nutrient tissue content of macroalgae may be the best option as this approach distinguished stress effects before the community it self was compromised. What we don't know from the current studies are the actual tipping points, e.g. when and unexpected change in the reef characteristics may generate a broad response in the system (Groffman et al. 2006). Certainly, this is an area where further research would be valuable. In particular it is important to get a good understanding of the baseline environmental conditions associated with the various reefs in this system - this will provide the context for management. I would then suggest that those reefs with i) the greatest level of background nutrient inputs and ii) lowest level of wave exposure be considered as a priority for monitoring. That monitoring of physiological responses in perennial

macroalgae be considered as this will provide a measure of the integrated impact response. The evaluation of responses during seasonal nutrient depletion in temperate reefs (spring/ summer) may act as reference values for the assessment of levels of response and to indicate potential spatial differences. Consequently, this should be done at the lowest temporal scale that is cost effective, but not less than seasonally. This also may be suitable to understand the variations associated with specie-specific internal nutrient pools. Where salmon farming is a key consideration, data about the size of the farming operation would be useful to determine relationships between potential changes in tissue nutrient levels and a given amount of nutrient loads in a given region. This may provide location-specific information about potential risk/ impact and thus establish empirical relationship on effects of nutrient loads. Similar approaches can be considered for land-based anthropogenic sources such as sewage treatment plants.

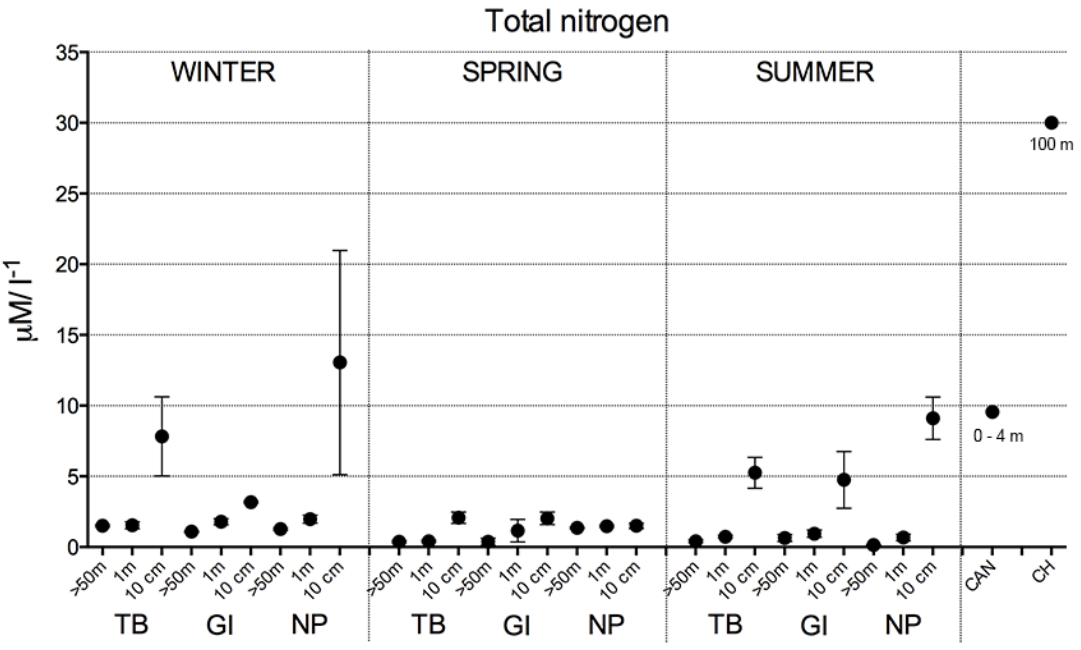

Figure 5. 1. Mean total nutrient concentration (± SE) at 1m and 10 cm from nutrient sources (Treatment plots) and >50 m (control plots) in three macroalgal reefs (TB = Tinderbox; GI = Green Island; NP = Ninepin Pt.) in the D'Entrecasteaux Channel during winter, spring and summer. Values correspond to NH_3+NOx+Phosphates concentration. Left panel shows mean values of nitrogen found next to commercial salmon farms in Canada (0 – 4 m form cages) and in Southern Chile (100 m from cages).

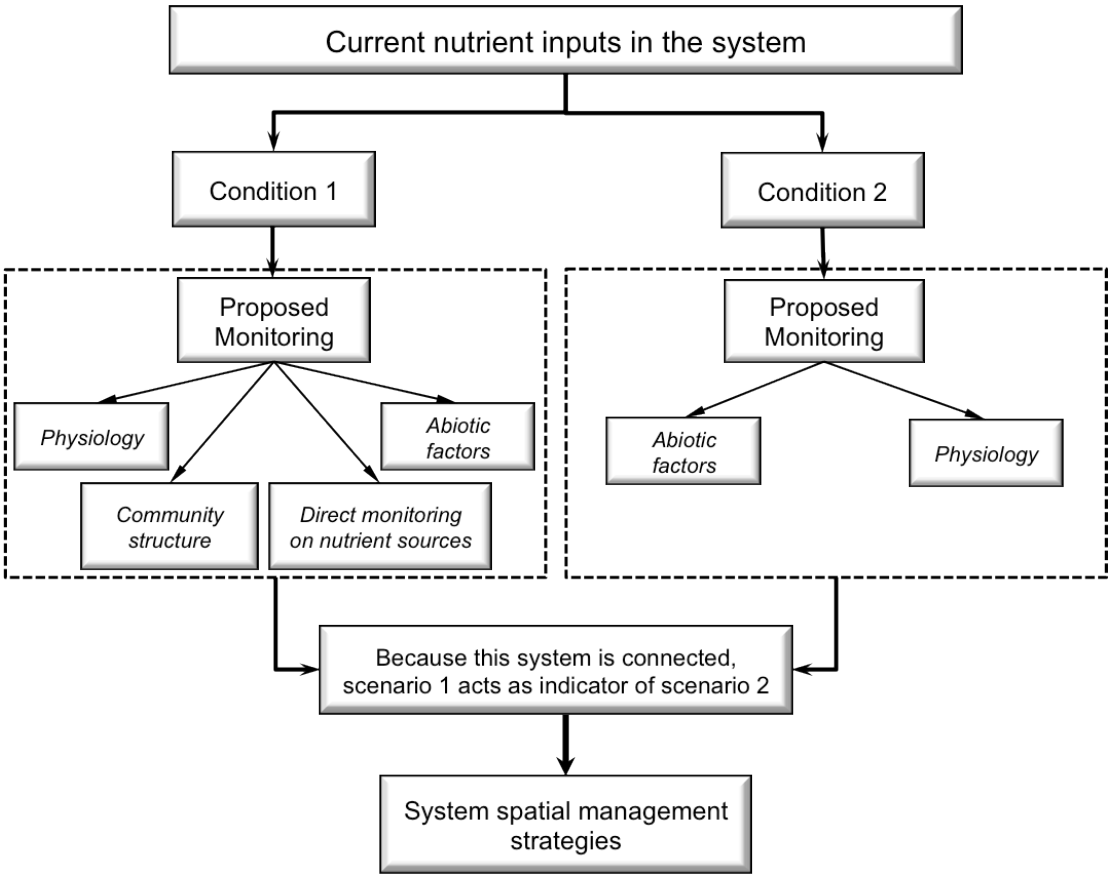

Figure 5. 2. Schematic framework outlining recommendation for two different approaches for monitoring and management based on the two different underpinning environmental conditions and community structures identified in this study i) Condition 1- the more sheltered location (Tinderbox); has greater background nutrient levels and more anthropogenic nutrient sources and the macroalgal community did not respond to seasonal nutrient fluctuations leading to the assumption that this system is nutrient saturated; this combination of circumstances was determined to require a higher level of monitoring as it is effectively the "canary in the coal mine" and may be more likely to show further stress responses to changes in the prevailing nutrient regime (e.g. shifts in community structure) and ii) Condition 2- the more exposed locations (Green Island/Ninepin Pt.); has lower levels of background nutrients and fewer anthropogenic nutrient sources, in this case the macroalgal community did respond to seasonal nutrient fluctuations, leading to the assumption that the system is unsaturated (under normal conditions); it was determined that any anomalous change would be evident based on physiological responses, thus this combination could support a lower level of monitoring as the risk of a significant stress to changes in community structure in the prevailing nutrient regime may be less. These scenarios and the proposed management responses are based on current levels of total nutrient inputs in the D'Entrecasteaux Channel.

5.8. Conclusion

The aim of this study was to clarify the response of rocky reef macroalgal communities habitats in southeast Tasmania to increased nutrient loads under different "real-world" environmental conditions and provide recommendations for management. The findings clearly showed that 1) at the level of impact occurring in these systems currently, and with the level of nutrient elevation attained with the field experiments there was no measurable effect on community structure and that the communities were able to readily adapt. Thus the reef systems may be somewhat resilient to moderate nutrient additions 2) changes were apparent at the physiological level suggesting that the plants were not completely unaffected by the additional nutrients. Moreover, there were differences observed between locations suggesting spatial variability in impact within the system, which in turn suggests that some prevailing local environmental conditions could ameliorate/ enhance impacts. This emphasized that the physiological response may pick up changes before threshold values are surpassed on rocky reefs (i.e., an early indicator of nutrient status). This has important implications for management. Monitoring approaches that account for location specific differences (i.e. physiological responses), physical variables such as wave exposure and light, and the associated algal community will likely be the most informative in determining effects of nutrient enrichment on macroalgal communities. Therefore different spatial scenarios may be considered for effective monitoring and management in estuarine systems.

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